Fibrocystic Disease of the Pancreas

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Report of the Eighteenth Ross PEDIATRIC RESEARCH CONFERENCE

Issued by Ross Laboratories (formerly M & R Laboratories)
Columbus 16, Ohio

The Eighteenth Ross Pediatric Research Conference, a symposium on Fibrocystic Disease of the Pancreas, was held under the auspices of the Department of Pediatrics of the State University of Iowa College of Medicine, at Iowa City, in the Conference Room of the Iowa University Hospital School for Severely Handicapped Children, September 30-October 1, 1955

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CHARLES D. MAY, M.D. — *Chairman, Department of Pediatrics State University of Iowa College of Medicine, Iowa City*

ROBERT G. FRAZIER, M.D. — *Assistant Professor, Department of Pediatrics, State University of Iowa College of Medicine, Iowa City*

Speakers

ERIKA BRUCK, M.D. — *Assistant Clinical Professor of Pediatrics, The University of Buffalo School of Medicine*

BARTON CHILDS, M.D. — *Assistant Professor of Pediatrics, The Johns Hopkins University School of Medicine, Baltimore*

ROBERT E. COOKE, M.D. — *Associate Professor of Pediatrics and Physiology, Yale University School of Medicine, New Haven*

PAUL A. DI SANT'AGNESE, M.D. — *Assistant Professor of Pediatrics Columbia University, College of Physicians and Surgeons, New York*

ZACHARIAS DISCHE, M.D. — *Associate Professor of Biochemistry Columbia University, College of Physicians and Surgeons, New York*

VERNON KNIGHT, M.D. — *Associate Professor in Medicine, Vanderbilt University School of Medicine, Nashville*

BENJAMIN H. LANDING, M.D. — *Assistant Professor of Pathology and Pediatrics, The Children's Hospital Research Foundation, Cincinnati*

CHARLES U. LOWE, M.D. — *Associate Professor of Pediatrics, The University of Buffalo School of Medicine*

STEPHEN W. ROYCE, JR., M.D. — *Instructor in Pediatrics, University of Southern California School of Medicine, Los Angeles*

HARRY SHWACHMAN, M.D. — *Assistant Professor of Pediatrics at the Children's Hospital, Harvard Medical School, Boston*

The purpose of these conferences is to assist in the correlation of the latest research information on specific topics of general interest in the practice of pediatrics, and to stimulate further research by the exchange of information These objectives coincide with the intention of the scientific staff of Ross Laboratories (formerly M & R Laboratories), manufacturers of Similac®, to keep informed of current developments in pediatric research It has become apparent that a large audience is interested in the conferences, and for this reason Ross Laboratories publish this report

The conference transcript has been edited and this report prepared by Robert G Frazier, M D Department of Pediatrics State University of Iowa College of Medicine

Printed in U S A.
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CLAUDE A. VILLEE, JR., Ph.D. — *Assistant Professor of Biological Chemistry, Harvard Medical School, Boston*

JOSEF WARKANY, M.D. — *Professor of Research Pediatrics, University of Cincinnati College of Medicine*

Participants

DOROTHY H. ANDERSEN, M.D. — *Associate Professor of Pathology, Columbia University, College of Physicians and Surgeons, New York*

GIULIO J. BARBERO, M.D. — *Assistant Professor in Pediatrics, University of Pennsylvania School of Medicine, Philadelphia*

HERBERT J. BARTELSTONE, D.D.S. — *Assistant Professor of Pharmacology, Columbia University, College of Physicians and Surgeons, New York*

EARL H. BAXTER, M.D. — *Chairman, Department of Pediatrics, The Ohio State University College of Medicine, Columbus*

SAMUEL P. BESSMAN, M.D. — *Associate Professor of Pediatrics, The University of Maryland School of Medicine, Baltimore*

JOHN M. CRAIG, M.D. — *Associate in Pathology at the Children's Hospital, Harvard Medical School, Boston*

GORDON E. GIBBS, M.D. — *Associate Professor of Pediatrics, University of Nebraska College of Medicine, Omaha*

LEE FORREST HILL, M.D. — *Chairman, Department of Pediatrics, Blank Memorial Hospital, Des Moines*

L. EMMETT HOLT, JR., M.D. — *Professor of Pediatrics New York University College of Medicine, New York*

DOUGLAS E. JOHNSTONE, M.D. — *Instructor in Pediatrics The University of Rochester, School of Medicine and Dentistry*

J. ALBRIGHT JONES, M.D. — *Assistant Professor of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia*

CHRIS P. KATSAMPES, M.D. — *Assistant Professor of Pediatrics, The University of Rochester, School of Medicine and Dentistry*

BENJAMIN KRAMER, M.D. — *Professor of Clinical Pediatrics New York University College of Medicine, New York*

RUSTIN McINTOSH, M.D. — *Carpentier Professor of Pediatrics, Columbia University, College of Physicians and Surgeons, New York*

PAUL R. PATTERSON, M.D. — *Chairman, Department of Pediatrics, Union University, Albany Medical College*

WYNNE SHARPLES, M.D. — *Chairman, Board of Trustees, Children's Exocrine Research Foundation, Philadelphia*

SAMUEL SPECTOR, M.D. — *Associate Professor of Pediatrics, Western Reserve University School of Medicine, Cleveland*

WOLF W. ZUELZER, M.D. — *Professor of Pediatric Research, Wayne University College of Medicine, Detroit*

"But concerning the sweat-bread, we declare nothing for a certainty, only we suspect that Obstructions, if not a Scirrhus, may sometimes invade that part. But this we delegate, to the enquiry of others."

Glisson, F., et al. *De Rachitide Sive Morbo Puerili, qui Vulgo the Rickets Dicitur, Tratatus* London (1650), cited in Ruhrah, J. *Pediatrics of the Past*, New York: Paul B. Hoeber, 1925

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Embryology as Related to Fibrocystic Disease of the Pancreas

Embryology of the pancreas and the parotid and submaxillary glands

Dr. Josef Warkany: In the fifth week of gestation, the 5 mm embryo shows the beginning of development of the pancreas by two outgrowths from the endodermal lining of the gut. At about the same time, the epithelial anlage of the respiratory system appears as an outgrowth of the pharyngoesophageal border. At six weeks the anlage of the parotid has also become discernible, and somewhat later the submaxillary gland makes its appearance.

During the seventh week, the 17 mm embryo shows fusion of the two pancreases into a single organ (figure 1.) The ventral pancreas forms the head, and its duct becomes the main outlet for the organ, the pancreatic duct of Wirsung. The dorsal duct becomes the accessory duct of Santorini.

At this time the epithelial lining of the duodenum, the esophagus and the rectum grows more vigorously than does the intestinal tube, resulting in the well-known temporary occlusion of these segments as indicated by the black bands (figure 1). Such physiologic occlusion of the lumen has not been found in the pancreatic ducts. The celiac plexus has now become recognizable in its prevertebral position

By 10 weeks of gestation histologic differentiation has begun. In the pancreas we see formation of acini, which originate as terminal or lateral buds from the ducts

The islands of Langerhans are present at 14 weeks, and in the second half of fetal life there develops a relative preponderance of islands compared to acinar tissue. The islands, like the acini, are products of differentiation from the ducts.

In the intestine, Lieberkuhn's crypts are well developed at 16 weeks, and Brunner's glands make their appearance during the sixth month. Meconium has reached the ileocolic junction at this time, however, it is not seen in the rectum before the end of the fifth fetal month Mucid cells are present in the salivary gland at 16 weeks Sweat glands become discernible as epithelial buds, yet they do not develop lumina before the seventh month of fetal life (figure 2).

The cells of Paneth, which occur in the human fetus not only in the crypts but also on the villi, do not appear before the seventh month. These cells contain about their nucleus large, acidophilic granules, which are discharged into the lumen under the influence of pilocarpine Zymogen granules of the pancreas react to pilocarpine in a similar manner.

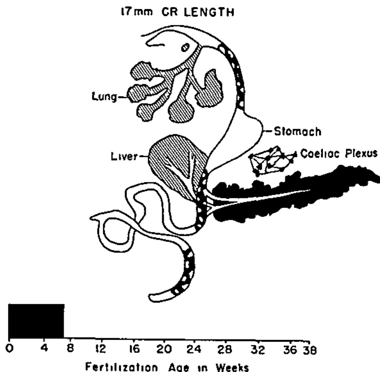


Figure 1 Development of organs involved in cystic fibrosis of the pancreas, by the seventh week of fetal life

Enzyme appearance

Trypsin can be found in the duodenum in the fourth month Pancreatic trypsin has been found in a few human fetuses at 16 weeks, but in two cases it was absent at 22 and 23 weeks. Pancreatic amylase has been found as early as 22 weeks, but it may be absent even at term. Pancreatic lipase is present at 32 weeks Proteolytic enzymes are produced by the pancreatic acini beginning with the fifth fetal month.

It seems that these enzymatic activities begin at about the midpoint of gestation. It is understandable that accurate data on this point cannot easily be gathered from human fetuses. However, a study has been made by Sampson of the changing quantity of trypsinogen in the pancreas of the fetal pig. The rapid development of the tryptic action, beginning in the seventh week and reaching almost adult activity in the tenth week, is shown in figure 3. Seven weeks of gestation represents the midpoint of pregnancy in the pig.

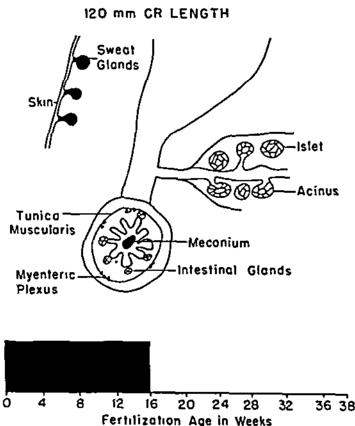


Figure 2. Development of exocrine glands in the sixteenth week of fetal life

The time at which pancreatic trypsin is found in the human fetus is in agreement with the measurement of trypsinogen activity in the fetal pig, indicating a parallelism in enzyme activation. It seems that pancreatic trypsin activity can be demonstrated soon after meconium appears in the intestine

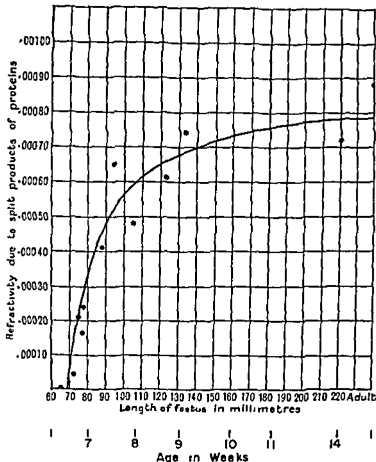


Figure 3 Development of tryptic activity in the pancreas of the fetal pig Reprinted from The Journal of Biological Chemistry, 38 345, 1919

Experimental pancreatic fibrosis

The progress made in experimental mammalian teratology during the past 15 years has not contributed further knowledge concerning the pathogenesis of crystic fibrosis of the pancreas Congenital anomalies of soft tissues can now be produced in rats and mice by a variety of teratogenic agents, but the pancreas is not among the organs so influenced

A deliberate effort to produce prenatal fibrosis of the pancreas was made by Lee, Kaplan, Wiseman and myself It has been shown that obliteration of the pancreatic acinar tissue, accompanied by

fibrous proliferation, can be produced by injection of dl-ethionine. These findings suggested the possibility of producing fibrosis of the pancreas in fetuses of pregnant rats by the injection of ethionine during pregnancy. Several variations of treatment and dosage were tried.

Although the ethionine treatment destroyed the mothers' pancreatic tissue, that of their fetuses grew and developed normally. Definite retardation of growth was observed in many litters.

It may be that not enough ethionine reached the fetal pancreas, or that the placenta changed the ethionine into a harmless material, or possibly, that ethionine is not effective because metabolism in the pancreas of the fetus is different from that in the adult animal. This experimental failure is regrettable, since the production of prenatal fibrosis of the pancreas would provide a useful tool for the investigation of fetal physiology.

Exocrine Function in Fetal Life

Dr Claude A. Villee: *During the past two decades,* research in biochemistry has done a great deal to elucidate the role of enzymes in the economy of the cell. As our ideas of the number and amount of these different enzymes have grown, it has become clear that most, if not all, of the protein material in a cell is actually enzymatic in nature. At the present time it would then be difficult to conceive of a living cell without these batteries of enzymes.

In each cell there are enzymes for the synthesis and breakdown of complex molecules of carbohydrate, protein, and lipid; for the intermediary metabolism of carbohydrates, fatty acids, and amino acids, for the release of energy from substrates and the transfer of this energy to substances such as adenosine triphosphate and other energy-rich compounds Each of these enzymes we now believe is regulated by a single gene Experiments in the field of biochemical genetics have established the so-called "one gene · one enzyme" theory as a sound working hypothesis.

*There is experimental evidence to support the concept that the fertilized egg has all or most of these enzymes, and that each daugh*ter cell receives its quota during the process of cell division By mechanisms quite unknown at present, additional enzyme molecules are synthesized within each cell so that the growing mass of cells is continually supplied with an adequate amount of metabolic machinery.

Enzymes essential for cell survival

Studies of the metabolism of human fetal tissue conducted in our laboratory have shown that the fundamental pathways of intermediary metabolism are well established at six to eight weeks of life in the human fetus. This is as early as we have been able to make observations Furthermore, these studies have shown that there is little change in the rates of activity along most of these metabolic pathways from then until term *The rates of oxygen consumption, glucose and pyruvate utilization, and lactate production are all rela*tively constant from eight weeks until term in fetal liver, heart, kidney, lung and cerebral cortex

Some changes in enzyme activity were observed in the course of these studies. For example, the enzymatic mechanisms for the synthesis of glycogen and the secretion of glucose are absent from

the liver until about the twelfth week of fetal life. Following their appearance they soon reach the adult level As development progresses, increase in the ability of the liver to produce glycine, to phosphorylate glycerol so it can be utilized, and to carry on transamination reactions was also observed

We have no comparable observations on the metabolism of exocrine glands, but there is no reason to doubt that, as development proceeds, the rates of oxygen consumption, glucose and pyruvate utilization, lactate production, and other intermediary metabolic reactions are relatively constant in these tissues also.

Specific enzymes

In addition to the enzymes that are common to all cells, there are others that are peculiar to specific tissues of the body. Examples of these are the many digestive enzymes produced by the salivary glands, the pancreas and the gastric and intestinal mucosa Secretion of such enzymes is not necessary for the survival of the cell.

In view of this, it is not surprising to find these enzymes absent when the tissue is first differentiated and appearing later in development. Investigators are not in complete agreement on when these enzymes can first be demonstrated in the human fetus. Needham lamented this fact in 1931, the same situation seems to exist today

Salivary amylase, or ptyalin, has been identified in the saliva of human fetuses as early as the fourth month of gestation The gastric mucosa of the fetus produces some pepsin, rennin and hydrochloric acid beginning with the fifth month. Trypsinogen and lipase are secreted in the pancreas by the fifth month But most investigators cannot demonstrate the presence of pancreatic amylase until birth or perhaps even shortly after birth

The enzymes produced by intestinal glands have been said by some investigators to be present as early as the fifth month, but this has been denied by others.

It has been reported that the gastric mucosa of the term infant is about eight times richer in pepsin than that of the premature infant The difference between term and premature infants in tryptic activity of the pancreatic extract is even more marked. It can be inferred from these results that the amount of pepsin and trypsin produced is much greater in the last month of gestation than earlier Their chief role before birth would appear to be that of digesting and liquefying meconium.

Nature of the biochemical lesion

The hereditary nature of fibrocystic disease of the pancreas suggests that it may belong to that class of diseases named inborn

errors of metabolism by Garrod in 1909. In these, one of the enzymes in a cell is altered, deficient or absent as the result of an action of a mutant gene. The disease would be comparable to alcaptonuria, albinism or glycogen storage disease of the liver.

The finding that trypsin is deficient or lacking would suggest at first glance that there is some impairment in the synthesis of this enzyme. However, pancreatic lipase and amylase are concurrently deficient or absent.

In all the other examples of inborn error, the change in a single hereditary unit or gene leads to the deficiency of a single enzyme. We might hypothesize that the action of this altered gene leads to some impairment in an enzyme system in the pancreas that is involved in a step common to the synthesis of all three enzymes. This would suggest that the primary biochemical lesion in fibrocystic disease is the result of a mutant gene which acts to decrease or to eliminate the ability of the pancreas to synthesize all three digestive enzymes. Meconium ileus would then occur because the enzymes are not present in amounts required to liquefy the meconium, and impaired digestion and absorption would follow. Still unexplained by this hypothesis are the viscid mucous congestion in the bronchial tree, and the atrophy of the acinar tissue of the pancreas.

A second hypothesis postulates that the mutant gene alters one of the enzymes involved in the production of mucoprotein secreted by the mucous glands. The action of the altered enzyme would cause the production of longer and more polymerized molecules than normal, resulting in a more viscid solution. This viscid mucus would lead to the plugging of the bronchial tree and of the ducts of the pancreas. The plugging of the pancreatic ducts would lead to atrophy of the acinar tissue and deficiency of pancreatic digestive enzymes, observations that can be made when the pancreatic duct is experimentally tied off.

It is consistent with this formulation that the deficiency of pancreatic enzyme is frequently not complete, and includes all of the pancreatic digestive enzymes.

Other clinical manifestations would follow from the decreased production of the pancreatic enzymes. The absence of lipase is probably the most important one, resulting in impaired digestion and absorption of fats, which would lead to the fatty liver sometimes observed, and particularly to the deficiencies of vitamins A and K, and cholesterol. The absorption of these fat soluble substances would be impaired along with that of lipids.

This latter theory of the etiology of the disease does not immediately suggest any new method of treatment, even if an abnormal molecular configuration of mucoprotein were found, for there is no

way known at present to delete or alter any of the intracellular enzymes of the body.

Discussion

DR. DOUGLAS E. JOHNSTONE: I would like clarification of the evidence that trypsin production in premature infants versus term infants is deficient

DR. VILLEE: Werner from Stockholm made pancreatic extracts and tested their tryptic activity; the differences were quite marked There was no overlapping of the ranges in activity of the extracts from term infants and from prematures.

DR L. EMMETT HOLT, JR: May and Vance did assays of duodenal contents and were not able to demonstrate a defect in the enzymes of the premature. Clement A. Smith made observations entirely in agreement with these, and quite different from those of Werner

DR HARRY SHWACHMAN: We have studied some prematures weighing 4½ to 5 lb and found approximately the same activity in the premature as in term infants. This has been verified indirectly by noting a rise in plasma amino acids following a whole protein feeding

DR JOHNSTONE: We have found that in a premature infant weighing 2 lb the first meconium plug had as much tryptic activity as that of any term infant we studied.

DR VILLEE: I don't think there is necessarily disagreement here The studies have been dealing with two different things, the amount of tryptic activity found in pancreatic extract, and the amount of tryptic activity found in duodenal juice The latter would probably be much more to the point, because it is the trypsin in the duodenal juice that is effective, not in the pancreas

DR ROBERT E. COOKE I should think, also, that analysis of the extract of the pancreas simply measures the amount present at any one moment, whereas in the synthetic process, the rate is probably the critical factor, and not the amount in the gland at any one moment.

DR DOROTHY H. ANDERSEN: I should like to inquire as to what data is available concerning the earliest age at which the pancreatic lesion of cystic fibrosis has been found at postmortem examination

In our experience, the earliest well-authenticated lesion appeared around the eighth fetal month. We have had a couple of premature infants who died of meconium ileus after having been

errors of metabolism by Garrod in 1909. In these, one of the enzymes in a cell is altered, deficient or absent as the result of an action of a mutant gene. The disease would be comparable to alcaptonuria, albinism or glycogen storage disease of the liver.

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This latter theory of the etiology of the disease does not immediately suggest any new method of treatment, even if an abnormal molecular configuration of mucoprotein were found, for there is no

had been in cardiac failure, with marked chest and gastrointestinal symptoms. With apparent clinical improvement, he no longer showed so high a concentration of iodide. These data on parotid secretion of iodide, I believe, contradict the possibility that there is a defect in energy production in secretory tissue.

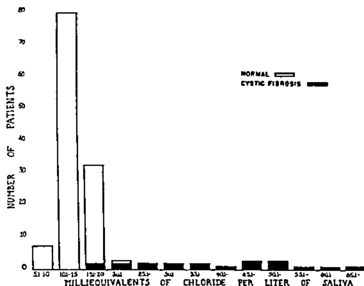


Figure 4. Concentration of chloride in the parotid secretion of children.

Dr. Cooke: I am not sure I would agree with that interpretation There are opposing arguments that can be based on the data on sweat. I do not think the calculation of free energy exchange can be based on the assumption that one anion determines the total work output of a gland We have some calculations for whole sweat; in these the chloride concentration at times actually runs inversely to the energy requirements for the whole secretion.

Dr Villee: I should like to ask whether the volume of saliva secreted by the two groups is the same

Dr Bessman: The volume is a little greater, on the average, in patients with fibrocystic disease than in the control children.

Dr Benjamin H. Landing: Can it be considered established that all exocrine glands are affected in this disease; or if not, can we arrive at a consensus about the glands with which we are concerned? This might give us additional information from which to construct hypotheses.

born in the eighth fetal month, and who had volvulus which must, by internal evidence have dated from about a month before

DR CHARLES U. LOWE: With reference to the secretin theory, is it possible that what is lacking is the stimulus to cause an elaboration of the three enzymes? This is a middle ground between the two possibilities proposed.

DR VILLEE: I don't see how the secretin theory can explain plugging in the bronchial tree

DR. GORDON E. GIBBS: In assays of the intestinal mucosa of patients who died, we have found normal secretin content

I was hoping to hear a third hypothesis, namely, a deficiency of some factor in the mechanism of secretion that would also involve the sweat glands.

DR. COOKE: That was a hypothesis that I was hoping to present, because I think there is another way that one might explain the concurrent findings in all the glands

DR. SAMUEL P. BESSMAN We have found that salivary secretion of chloride is not at all affected by secretin. Administration of secretin to normal children and to patients with fibrocystic disease of the pancreas did not affect the concentrating power of the salivary gland in either group

Perhaps there is a basic lesion of secretory tissue in general that could cause all these abnormalities. We started with the assumption that there might be a defective mechanism for energy production by secretory tissue, that the adenosine triphosphate generation was abnormal in these glands.

There is a significant difference in parotid secretion of chloride, as there is in the sweat, between normal controls and patients with cystic fibrosis of the pancreas We have evidence suggesting that there is no energy for the retention of chloride, for keeping it in the blood, and in the patient with this disease it leaks into the parotid and other exocrine secretions Figure 4 shows the concentration of chloride in the parotid secretion of normal children (135) and those with cystic fibrosis of the pancreas (20)

There is another ion secreted by the parotid gland that is interesting in this respect—it is iodide In the normal individual the concentration of iodide in the parotid saliva is between ten- and thirty-fold greater than the blood concentration

We gave iodide to a large number of normal children and to four patients with cystic fibrosis of the pancreas, and measured the parotid secretion of iodide following this administration Figure 5 shows that these patients secrete iodide in higher concentration than do the normal individuals

The triangles in this figure represent the iodide secretion values obtained in a patient after about three weeks of therapy This patient

Dr. Andersen: I should like to point out that in the glands on which there is general agreement regarding involvement, i.e. pancreas, intestinal glands, sweat glands, salivary glands, respiratory epithelium and biliary epithelium, there are three kinds of abnormalities.

1) morphologic concretions within the glands and ducts, as in pancreas, liver, and intestional glands;

2) dilatation of the glands and ducts with excess secretion, but without actual concretions, or very rare ones as in the bronchial mucous glands and Brunner's glands In these the glandular secretion appears, histologically, different from what is seen in the pancreas;

3) chemical abnormality of the secretion without associated morphological changes. as in the sweat glands, where under the microscope nothing abnormal is seen. and a chemist is required to discover the abnormality.

In view of three different types of change. any theory based on study of only one gland is probably inadequate.

DR. ANDERSEN: Are all agreed there is something wrong with the pancreas?

DR. DI SANT'AGNESE: All of us agree that there are some patients in whom the pancreas functions normally. Whether these patients have some involvement of the pancreas that is not recognizable clinically, because the methods are too gross, is something we cannot say.

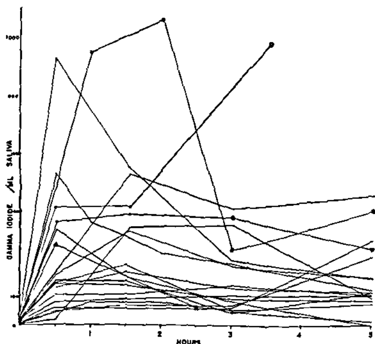


Figure 5. Excretion of iodide in parotid saliva following oral administration of potassium iodide. Large dots are values from patients with fibrocystic disease of the pancreas.

DR. ANDERSEN: What proof is there of the disease pathologically, if the pancreas is normal?

DR. WOLF W. ZUELZER: That is very much to the point, we have no proof. I think all of us have occasionally seen lesions in the respiratory tract simulating closely, if not indistinguishable from, the pulmonary lesion seen in pancreatic fibrosis, without being able to demonstrate lesions in the pancreas. Pathologically, we have no way of knowing whether they are related to this disease.

Among the living, case 4 has had no recurrence of gastrointestinal bleeding since operation, a period of three years, but the pulmonary status has deteriorated. Cases 2 and 6 are doing only moderately well from the pulmonary standpoint, but have not been greatly troubled by liver involvement Case 7 is doing well from all standpoints, although recently hypersplenism has begun to appear (table 1).

Table I

Data on seven patients with fibrocystic disease and cirrhosis of liver

Case	Hepato-splenomegaly	Liver biopsy	Liver function tests	Portal system pressure*	Hypersplenism	G I bleeding	Ascites	Shunting operation
Group I								
1	+	Cirrh	N	370mm	+	+	0	+
2	+	Cirrh	+	N D	+	0	?	0
3	+	Cirrh	+	450mm	+	+	+	+
4	+	Cirrh	+	570mm	+	+	+	+
Group II								
5	+	N D **	N	N D	0	+	0	0
6	+	N D.	+	N D.	0	?	0	0
7	+	N D	N	N D	+	0	0	0

* Normal = less than 200 mm of water
** N D. = not done

In all patients, hepatosplenomegaly was present. The liver was hard, nontender, and moderately enlarged, and its surface irregular; in several instances, nodules were felt. The spleen was palpated in most cases 3 to 5 cm below the costal margin, firm, nontender, and smooth In case 3, the spleen was enormously enlarged and occupied much of the left side of the abdomen. In the first four cases, biopsy of the liver revealed cirrhosis. Jaundice was absent in all.

Although the symptoms of hypersplenism, gastrointestinal bleeding or ascites or a combination of the three, were much more severe in Group I, all the patients presented evidence of portal hypertension On exploratory laparatomy in cases 1, 3 and 4, the pressure in the portal system was found to be markedly increased A surgical shunting procedure to relieve the increased tension was performed in these cases

Pathologic changes

The variations in the degree of cirrhosis, with the more severe lesions chiefly in the older children, offer an opportunity for study

Fibrocystic Disease of the Pancreas with Cirrhosis of the Liver

DR PAUL A DI SANT'AGNESE: Review of the medical literature indicates that hepatic changes described in patients with fibrocystic disease have been largely chance findings at autopsy, giving rise in the majority of cases to no clinical symptoms. It can be shown, however, that in patients with fibrocystic disease of the pancreas the initial lesions may progress to severe and, at times, fatal clinical manifestations, due to a distinctive type of multilobular biliary cirrhosis with concretions. The studies summarized here were carried out in collaboration with Blanc.

Clinical material studied

Data on 25 patients with fibrocystic disease show that, in the absence of clinical evidence of liver involvement, chemical determinations related to hepatic function are normal

Seven patients have been observed with fibrocystic disease of the pancreas and clinically manifest cirrhosis of the liver They *have been divided into two groups Group I, four patients, with* biopsy of the liver; and Group II, three patients in whom hepatic biopsy was not performed, but in whom the diagnosis of biliary cirrhosis was felt to be established on clinical grounds.

When involvement of the liver was first diagnosed, the patients ranged in age from 4 to 10 years *All seven patients had pulmonary* disease characterized by generalized obstructive emphysema and chronic bronchopneumonia in varying degrees of severity In cases 1 and 4, this had not been present when the hepatic cirrhosis was diagnosed In all seven cases the concentration of sodium and chloride in the sweat was increased *Five of the seven had pancreatic* deficiency Duodenal content in case 4 was extremely viscous, but had a level of tryptic activity in the low normal range. In case 2, tryptic activity of material obtained on duodenal assay was normal at first, but later decreased to very low levels

Of the seven patients, three are now dead Cases 1 and 5 died of progressive pulmonary disease, which in case 1 had not been present at the time of operation. Case 3 died of uncontrollable gastrointestinal hemorrhage.

Among the living, case 4 has had no recurrence of gastrointestinal bleeding since operation, a period of three years, but the pulmonary status has deteriorated. Cases 2 and 6 are doing only moderately well from the pulmonary standpoint, but have not been greatly troubled by liver involvement. Case 7 is doing well from all standpoints, although recently hypersplenism has begun to appear (table 1).

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2	+	Cirrh	+	N D.	+	0	?	0
3	+	Cirrh	+	450mm	+	+	+	+
4	+	Cirrh	+	570mm	+	+	+	+
Group II								
5	+	N D **	N	N D	0	+	0	0
6	+	N D	+	N D	0	?	0	0
7	+	N D.	N	N D	+	0	0	0

* Normal = less than 200 mm of water
** N D. = not done

In all patients, hepatosplenomegaly was present. The liver was hard, nontender, and moderately enlarged, and its surface irregular; in several instances, nodules were felt The spleen was palpated in most cases 3 to 5 cm below the costal margin, firm, nontender, and smooth. In case 3, the spleen was enormously enlarged and occupied much of the left side of the abdomen. In the first four cases, biopsy of the liver revealed cirrhosis. Jaundice was absent in all

Although the symptoms of hypersplenism, gastrointestinal bleeding or ascites or a combination of the three, were much more severe in Group I, all the patients presented evidence of portal hypertension On exploratory laparatomy in cases 1, 3 and 4, the pressure in the portal system was found to be markedly increased A surgical shunting procedure to relieve the increased tension was performed in these cases.

Pathologic changes

The variations in the degree of *cirrhosis*, with the more severe lesions chiefly in the older children, offer an opportunity for study

of the evolution of the hepatic lesions. The earliest changes seen in our series of patients with cystic fibrosis of the pancreas were noted at three days of age.

Focal biliary cirrhosis with concretions. On inspection of the capsule at autopsy, the initial lesion is seen as a varying degree of pitting, corresponding to small, stellate, depressed cirrhotic foci.

Microscopically, this focal biliary cirrhosis with concretions is characterized by biliary proliferation, inflammatory reaction, concretions of amorphous eosinophilic material plugging the bile ductules, and absence of marked bile stasis. The nature of the concretions is not clear, but they show a morphologic and histochemical resemblance to those in the pancreas. Both may be interpreted as resulting from inspissated secretions. The concretions in either organ react negatively to Feulgen's test for nucleic acid, stain similarly with periodic acid-Schiff reagent, do not show metachromasia, *and have a similar pH curve by the methylene blue extinction test.*

Multilobular biliary cirrhosis with concretions. With time, multiple foci merge. Diffuse portal changes take place. Multiple lobules are encircled and trapped by the fibrotic process.

As fibrosis becames more extensive, the architecture is destroyed and the original foci of concretions may entirely disappear, although, more commonly, occasional foci of eosinophilic concretions are found. Because of the focal character of the initial lesions, damage proceeds irregularly, and areas of preserved lobular pattern are found adjacent to massive foci of bile duct proliferation with fibrosis.

This multilobular biliary cirrhosis is followed by portal lesions and ends in remodeling of hepatic architecture with regenerative nodules. Typical of this kind of cirrhosis is the formation of irregular nodules larger than those in the usual type of portal and biliary cirrhosis. Retraction of fibrous tissue leads at times to deep clefts producing a hepar lobatum. Characteristic also is the absence or limited degree of bile stasis.

At this late stage only, the spleen is enlarged and shows the chronic passive congestion with pulp cord fibrosis typical of portal hypertension.

The name of multilobular biliary cirrhosis with concretions has been chosen as *descriptive of these advanced hepatic lesions, because* a group of liver lobules is involved in the process from the begin-
. monolobular biliary cirrhosis,
of cholestatic or cholangitic
iary, cirrhosis may also result

from massive necrosis with collapse of the collagenous framework of the liver. Use of the terms *multilobular, biliary* and *concretions* expresses the distinctive character of the cirrhosis in fibrocystic disease of the pancreas.

Development of the clinical picture

The first lesion is a focal biliary cirrhosis which gives rise to no clinical manifestations and is a chance finding at postmortem examination. Even when the lesions are extensive, their scattered character is such that, though a significant amount of hepatic parenchyma is affected, there is no clinical or laboratory evidence of involvement of the liver.

In some instances the process becomes generalized Such a patient goes on to a diffuse and severe hepatic cirrhosis, which combines scarring and regenerative nodules with diffuse portal changes. On physical examination, the liver is found to be moderately enlarged, hard, nontender, frequently nodular. An unusual feature is the apparent variation in the size of this organ as it descends further into the abdomen or recedes with each cycle of pulmonary infection, as a consequence of the differences in mechanical pressure exerted through the diaphragm by alternation in the degree of distention of the lungs. Chemical tests of liver function and serum bilirubin are still normal

After a varying length of time, a few months or a few years, the distortion and remodeling of the architecture of the parenchyma of the liver is such that portal hypertension eventually develops. It is at this stage that the condition becomes clinically manifest,

[illegible]

rubin is either normal or very slightly increased.

Incidence

The autopsy material for the twenty years, 1935-1955, was reviewed in an attempt to establish the incidence of hepatic lesions in this disease. Twenty-five of 116 patients with fibrocystic disease of the pancreas at Babies Hospital had cirrhotic lesions at autopsy, an incidence of 22 percent Sixteen of these had single or multiple lesions of focal biliary cirrhosis In nine, the changes were more extensive.

Cirrhosis of the liver, with portal hypertension, has been observed in 7 of 311 patients with fibrocystic disease of the pancreas, an incidence of a little more than 2 percent.

A survey of the diagnostic files from 1949 through 1954 shows that hepatic cirrhosis was entered among the final diagnoses in 28

cases. Seven of the patients had fibrocystic disease of the pancreas; in 10 others the cirrhosis of the liver was of unknown origin; in seven, atresia of the bile ducts was the basic disease; in two, chronic acquired hemolytic anemia; in one, glycogen storage disease of an unusual type, and in one, schistosomiasis. Portal hypertension was present in 11 of the 28

In our experience, therefore, a child with the syndrome of portal hypertension secondary to cirrhosis of the liver has a good chance of having fibrocystic disease of the pancreas. The concentration of electrolytes in the sweat has been found to be characteristically increased above normal only in patients with fibrocystic disease, and the test is therefore of value in the differential diagnosis of cirrhosis.

Etiology

The histologic appearance suggests that the cycle is initiated by primary mechanical obstruction of bile ductules by what appear to be inspissated secretions, an expression presumably of the abnormality of secretory products of exocrine glands that is characteristic of the disease.

While focal biliary fibrosis is a common postmortem finding in patients with fibrocystic disease of the pancreas, the diffuse multilobular biliary cirrhosis with clinical manifestations is rare. What, then, triggers the passage from one type of hepatic lesion to the other?

A varying degree of involvement of the affected area is characteristic of fibrocystic disease of the pancreas. In four of the patients described, the pulmonary disease was milder than average In four, the concentration of electrolytes in the sweat was abnormally elevated but in the lower range of the values characteristic of this condition. In two, pancreatic function was either within normal limits or normal at first, with deficiency appearing later

It is possible, therefore, that patients with cystic fibrosis who develop cirrhosis of the liver provide another example of the variability of this disease It might be postulated that an added infection, e g., infectious hepatitis or nutritional insult, could cause an adverse response by a liver which is already basically abnormal. It is difficult to incriminate any single factor in the elicitation of this response.

Nutritional deficiency, ascending cholangitis in a partially obstructed biliary tree or toxicity from long continued administration of antibiotic drugs might all be postulated and may be present or absent in an individual. Certain it is that diffuse and clinically manifest hepatic cirrhosis in fibrocystic disease of the pancreas was rare some years ago, but is being recognized with increasing frequency now that survival of patients is greater The condition does

not occur, however, in young children, and it must be assumed that the main effect of age is longer exposure to a noxious agent and, consequently, increased chance of developing hepatic lesions in a progressive disease.

It is too soon to try to prognosticate for patients who develop cirrhosis of the liver with portal hypertension, but current observation would indicate that it is not impairment of hepatic function, but rather increased tension in the portal system and consequent gastrointestinal bleeding that represents their main hazard. Surgical shunting procedures are the patient's best hope for survival, and should be undertaken if the severity of the bleeding or, at times, the degree of hypersplenism points to the necessity for surgical intervention.

The outlook for patients surviving operation in which portal pressure is successfully relieved is still not well-defined.

Summary

Another major area, the hepatic, must be added to those already known to be frequently involved in this disease. The localized and focal hepatic lesions, presumably resulting from primary mechanical obstruction, may progress to a pathologically distinct and widespread multilobular biliary cirrhosis with concretions. Clinically, this advanced cirrhotic process manifests itself in patients with fibrocystic disease of the pancreas by hepatosplenomegaly, and is characterized by the symptoms of portal hypertension and, usually, an absence of icterus.

Discussion

Dr. Vernon Knight. In patients with acute respiratory infection, where the liver comes down in the course of infection, is there an associated period of abnormal function of the liver?

Dr di Sant'Agnese. As far as I know, chemical tests of hepatic function show no changes under such circumstances. Mechanical factors seem to be largely responsible for the apparent enlargement.

Dr Shwachman: I should like to ask whether dietary or other therapy can be related to the severity of the cirrhosis. We have encountered cirrhosis of the liver less frequently, nor do I recall ever seeing a child with portal hypertension, secondary to the hepatic changes in fibrocystic disease.

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of a child with hepatic disease from other cause; is this pathology specific before the end stage?

DR. ANDERSEN: I think that the microscopic V-shaped lesions beneath the capsule are specific. I have never seen anything quite like this in any other disease. Some of the later, severe cirrhotic lesions are complex and I can not always be sure of their origin. In our experience, the diagnosis of fibrocystic disease has been made in several patients, of a type and degree that has required operation for portal hypertension.

DR. SHWACHMAN: Do you think biopsy on someone who is not undergoing surgery is justified?

DR. DI SANT'AGNESE: We are not justified in performing a biopsy if patients have no symptoms of gastrointestinal bleeding. If severe gastrointestinal bleeding is present, I think we are obligated to undertake surgery to relieve portal hypertension and then a biopsy of the liver should be obtained.

DR. KNIGHT: If there were assumed a superimposed lesion on the liver that might precipitate further disturbance and progression of cirrhosis, infectious hepatitis should be considered. The fact that we can use gamma globulin to alleviate the disease prophylactically indicates that children can have it at a very young age in subclinical form.

I wonder if the patient who already has focal lesions of the liver might suffer more damage from infectious hepatitis than the patient without such lesions.

DR. ANDERSEN: It would seem quite likely, but rather hard to test objectively.

DR. DI SANT'AGNESE: Once portal hypertension starts it seems to develop very rapidly, although the precipitating mechanism is not clear.

DR. CHARLES D. MAY: The recognition may be abrupt and the sequence of events rapid. We have not actually succeeded in isolating, from scrutiny of the histories, events which might be looked upon as precipitating.

DR. BARBERO: An analogy, regarding the precipitation of more diffuse liver pathology, may be provided by information we obtained from study of the Chinese liver fluke, Clonorchis sinensis. This parasite produces a biliary type of cirrhosis, cholgangitis, and inflammatory involvement. We found that these children in the Orient can live in a symbiotic relation with the disease.

As nutrition deteriorated where lack of food occurred, the children developed intense liver disease, became jaundiced, and showed progressive and massive enlargement of the liver. This condition was reversed as nutrition improved.

DR. DI SANT'AGNESE: No definitive answers can be given because the number of patients is small. Two of our patients did not have any clinical signs of pancreatic deficiency when the cirrhosis with portal hypertension was manifest. Two others did not have much in the way of pulmonary infection, but had been diagnosed as having fibrocystic disease and kept on restricted diets for many years before the cirrhosis of the liver appeared. Dietary deficiency, therefore, may have an effect in some but not all patients.

DR. GIULIO J. BARBERO: Two of our six patients with biliary cirrhosis first came in without evidence of pancreatic disease and with only minimal evidence of pulmonary disease; they both had portal hypertension and nodular livers. We have had identical twins who, very early in life, developed nodular livers which have progressively enlarged. They have shown signs of some degree of portal hypertension and hypersplenism, later developing signs of pancreatic deficiency, and now manifesting advanced pulmonary disease.

DR. JOHN M. CRAIG: Of 184 instances of cystic fibrosis of the pancreas coming to autopsy, we have 10 that we have labeled focal biliary cirrhosis. In only two of these was there obvious bile stasis, and in none was there evidence of portal hypertension. We agree with Zuelzer that these cases of focal biliary cirrhosis are unusual and specific ones. The regenerative nodules that di Sant'Agnese describes have not been found.

The histological pictures of two of the cases that di Sant'Agnese reports here suggest that some other lesion has been superimposed on the liver of a patient with cystic fibrosis of the pancreas.

In reviewing our pathology records from 1920 until the present, we found the age at death of these patients is progressively increasing, and thus we can expect a different pathologic picture over the years.

increasing age, and most of the periportal fibrosis is accompanied by a hyperplasia of the bile ducts leading to focal biliary cirrhosis. As di Sant'Agnese suggested, this hyperplasia of the bile ducts is probably secondary to the inspissation of bile in the ducts.

The nutritional status at the time of autopsy cannot be correlated with any particular change in the histology of the liver. We do not subscribe to the published thesis that the nutritional status in cystic fibrosis of the pancreas is particularly important in the development of cirrhosis.

DR. LOWE: Is it possible in the early stages of this lesion to differentiate a liver from a child with fibrocystic disease from that

are some myoepithelial cells which are scattered rather irregularly, and are thought to be able to contract, expel fluid, and contribute to the initial secretion, at least by forcing out material already in the duct. They may contribute to the secretion pressure, said to be as high as 250 mm of mercury. There is a separate blood supply for the secretory portion as contrasted with the tubular portion; I do not know the significance of this.

Histochemistry

Histochemical study of the sweat glands has revealed certain metabolic aspects which may be of importance. The secretory portion of the gland is faintly basophilic or acidophilic in staining characteristics, and contains many lipid and glycogen vacuoles. The lack of metachromatic staining is suggestive evidence that acid mucopolysaccharides are absent The presence of abundant glycerophosphatase and of alkaline phosphatase, and the absence of acid phosphatase has been noted in eccrine sweat glands. It is of interest that these glands contain large amounts of glycogen, both in secretory and duct cells. By contrast, apocrine glands contain no glycogen, much less alkaline phosphatase, and some iron.

The abundant glycogen in the secretory cells at the base of the eccrine sweat glands disappears with activity, suggesting that the glycogen may be used for energy for secretion. The role of the alkaline phosphatase has not yet been determined

The distribution of sweat glands in various parts of the body is of some importance with regard to the practical measurement of sweat for diagnostic purposes. The iodine starch spot technique has demonstrated that the number of sweat pores varies considerably from one part of the body to another. The sweat pore count ranges from 350 on the dorsum of the hand down to 150 on the anterior chest

It has further been observed that during moderate thermal sweating there is a cyclic discharge by larger and smaller numbers of sweat glands With increased stimulation each sweat gland puts out a larger amount of fluid. Thermal sweating is confined primarily to the trunk, extremities and head. The glands of the palms of the hands the soles of the feet and the axilla are primarily activated by emotion

Pharmacology

The innervation of the sweat glands has been demonstrated to be through the thoracolumbar outflow of the sympathetic nervous system Of significance, for our purposes is a consideration of the chemical mediator at the end of the postganglionic sympathetic

Sweat — Basic Physiology

Anatomy

DR. ROBERT E COOKE· Sweat glands fall into two general groups: eccrine glands which are present over most of the body surface, and the apocrine glands which are present primarily in the axilla and perianal region, the latter beginning to function at puberty. Most of this discussion will be devoted to the physiology of the eccrine glands.

Grossly, the sweat gland may be divided into two portions, a secretory portion which has been called a glomerulus by analogy to the kidney, and a tubular portion which has been likened to the tubules of the kidney (figure 6). The analogy of the glomerulus is

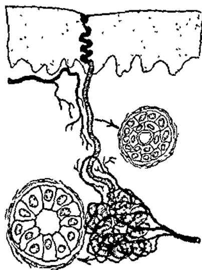


Figure 6. Schematic drawing of the anatomy of the sweat gland Reprinted from Archives of Dermatology and Syphilology 57 907, 1948

inexact in that there is no known filtration mechanism in the sweat gland. The secretory portion consists of a coiled duct made up of a single layer of cuboidal cells. The collecting duct, on the other hand, is a double-layered tube, with a very dense, thick, cuticular inner border In the small *intercellular canals of the latter there*

Flushing of the sweat gland is of some importance, since after two or three periods of sweating, sweat concentrations of sodium and chloride fall to a stable level. Potassium concentration falls considerably. This phenomenon has been described as a washing out of retained concentrated sweat from the ducts

Table 2

Factors decreasing the concentration of sodium and chloride in sweat

Systemic	Local
Relative hyperadrenalism	Low skin temperature
DCA, ACTH	Acclimatization
Adrenal tumor	Flushing of gland
Hyperthyroidism	
Increased K intake	
Na deficit	
Acclimatization	
Repeated muscular work	

Table 3

Factors increasing the concentration of sodium and chloride in sweat

Systemic	Local
Relative hypoadrenalism	Region—especially palmar
Addison's Disease	Vapor barrier
Panhypopituitarism	Skin temperature
High NaCl intake	Increased rate
Elevation of rectal temperature	Prolonged sweating (fatigue)
Elevation of deep skin temperature	Arterial occlusion
	CYSTIC FIBROSIS

The systemic factors that cause an elevation of sodium and chloride concentrations in sweat are, in part, the converse of those previously listed (table 3). Elevation of rectal temperature and of deep skin temperature may express themselves through their effects on rate of sweating, which I have listed among the local factors

Interpretation of sweat tests for diagnostic purposes must be approached with some caution. The concentrations of electrolyte

fibers to the eccrine sweat glands; this has been demonstrated to be acetylcholine

The sweat gland responds to drugs with muscarinic effect, such as pilocarpine or mecholyl, when injected intradermally. This response is blocked by atropine.

By the use of a staining technique, it has recently been shown that specific cholinesterase is present in abundance around the secretory cells of eccrine glands, thereby indicating their cholinergic innervation By contrast, no specific cholinesterase could be demonstrated around the apocrine sweat glands.

Even though a small amount of sweat may be produced by local administration of adrenergic drugs, there is no evidence for adrenergic innervation of the sweat glands. Almost certainly the chemical transmitter is acetylcholine, thus putting the sweat gland in the same category with mucous glands, salivary glands, and the pancreas.

We have attempted to measure the adrenergic sweating, but the amount is so small that quantitative measurements are impossible. It is our opinion that epinephrine may stimulate myoepithelial elements leading merely to evacuation of the ducts and not to true sweat formation The evidence for this effect in the apocrine sweat glands is excellent, for eccrine sweat glands, less convincing

Factors affecting electrolyte composition

With relation to fibrocystic disease, an important aspect of the physiology of sweat glands is a study of the factors influencing the composition of sweat The water, sodium, potassium, chloride, lactate, bicarbonate and urea content, and the pH of sweat have been studied Lactate in sweat may have bearing on the provision of energy for secretion, but little is known about it

Of greatest import to the problem of cystic fibrosis is a consideration of the factors that influence the sodium and chloride concentrations in sweat

Factors decreasing the concentration of sodium or chloride in sweat may be divided somewhat arbitrarily between systemic and local, as in table 2. An increase of salt-and-water hormone or exogenous DOCA-like steroids reduces markedly the concentration of sodium and chloride in the sweat It is likely that the significantly lower concentration of sodium in sweat in severe hyperthyroidism, as contrasted with that of mild hyperthyroidism or euthyroidism, is due to the relative hyperadrenocorticism that accompanies the former The action of potassium excess, sodium deficit and acclimatization may be mediated through an increased output of adrenal steroids In acclimatization, hyperadrenalism is only one of the factors concerned

microEq/M²/min against the rate of flow of sweat, the curve shown in figure 8 is obtained. There is a break in the curve, and above a flow rate of 5 gm/M²/min sodium excretion is proportional to sweat flow. The regression equation for the line, for rates greater than 5 gm/M²/min, shows the intercept of this line not at zero but at about −185 microEq/M²/min. This quantity was assumed to be the amount of sodium reabsorbed from the original precursor solution per square meter per minute. The slope of that line, 68 6, actually represents the number of microEq of sodium put into each gram of final sweat. The line for potassium intersects zero, indicating no reabsorption.

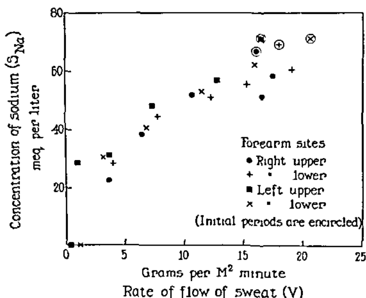


Figure 7 Relation of concentration of sodium in sweat to rate of flow of sweat. Reprinted from The Journal of Clinical Investigation, 35 114, 1956

Conditions of low sodium intake are associated with low sodium concentrations in the sweat With low concentrations of sodium in the sweat, there may actually be less put into the precursor solution per unit of time, and considerably less reabsorbed The argument that low sweat electrolyte concentrations are due to increased reabsorption of electrolyte are not substantiated by these studies

Data on various secretions are shown in table 4. The arrows indicate a rise in concentration with rate of sweating

vary considerably from one region of the body to another. Palmar and axillary sweat have very high sodium chloride concentrations, especially with intermittent sweating. In a study on the relationship between whole body sweat and sweat collected under a vapor barrier, it was found that the concentration of electrolyte collected locally under a vapor barrier is always higher than in whole body sweat, the differences being less marked at lower electrolyte concentrations Whether these findings are due in part to higher skin temperature and/or increased local rate must be determined In addition, it is possible that some back diffusion of water might elevate concentrations.

Prolonged sweating also leads to a rise in concentration as well as an occasional fall in rate. Exactly the same changes have been observed after arterial occlusion

Neither excess water intake nor acute elevation of the plasma sodium and chloride concentrations with intravenous hypertonic salt affect the composition of sweat. Pitressin, which increases water reabsorption in the renal tubule, probably does not affect water reabsorption from the sweat duct, since electrolyte and nitrogen concentrations are not altered

Our experiments have corroborated findings to the effect that, with increasing skin temperature, there is a rise in chloride concentration to quite a marked degree, and sodium concentrations rise proportionately. The volume of sweat rises less acutely with increasing skin temperature than does the concentration of chloride. In sweating induced by cholinergic drugs such as mecholyl, there is less effect from changing skin temperature.

The marked change in electrolyte concentration in one study on the effect of rate of sweating is shown in figure 7. As the rate of sweating increases, there is an increase in sodium concentration tending to approach a maximum of 56 mEq/l The concentration of sodium in the saliva also rises with increasing rate of flow

Process of sweat formation

Earlier work suggests that a fairly constant precursor solution of sweat is elaborated and as the secretion passes through the tubule, water and sodium may be reabsorbed Changes in sodium concentration of sweat have been attributed to changes in the reabsorption of sodium by some portion of the sweat gland.

Such theoretical considerations are of great importance with regard to the problem of cystic fibrosis There has been an attempt to give some mathematical expression to this reabsorptive phenomenon and to the total excretory phenomenon by the secretory cells of the sweat glands. By plotting the rate of excretion of sodium in

These data suggest that the precursor solution has a high concentration of sodium when the concentration of sodium in the sweat is high and a low concentration of sodium when the concentration of sodium in the sweat is low, and that, consequently, reabsorption plays a minor role in determining the concentration of sodium in the sweat.

Physiology of sweat in fibrocystic disease

It was of some interest to us to determine whether the rate of sweating might be increased in patients with cystic fibrosis. Table 5 shows there is little difference between these patients and a control

Table 4

Composition of exocrine secretions in normal individuals

	[Urea] Secretion / [Urea] Plasma	Concentration mEq/l		
		Na	Cl	K
Tears	1 02	140	125	16
Saliva	?	20 → 90	10 → 43	20
Sweat	1 75	20 → 56	10 → 40	9

Table 5

Volume of sweat

Units	Controls	Patients with cystic fibrosis
ml/M²/hr	144	152
ml/100 cal	211	274
Respiratory Water Loss		
gm/kg/hr	622	636

group The caloric turnover for some of the control subjects who tended to hyperventilate because they were not accustomed to aerosol equipment may be high. Respiratory water loss was about the same in the two groups

Three possible explanations for high concentration of sodium in the sweat of patients with cystic fibrosis are greater water reabsorption by the ducts of the sweat glands, less sodium reabsorption or high sodium concentration in the precusor solution.

It is apparent that urea is concentrated in the sweat of normal individuals. Urea, a very freely diffusable molecule, is assumed to enter the precursor solution by passive diffusion. Then, as water is reabsorbed, the urea concentration rises. With increase in the rate of flow, the ratio of the concentration of urea in sweat to that in plasma has been found to remain quite constant at about 1.75 whereas concentrations of sodium and chloride rise with increase in rate of flow of sweat.

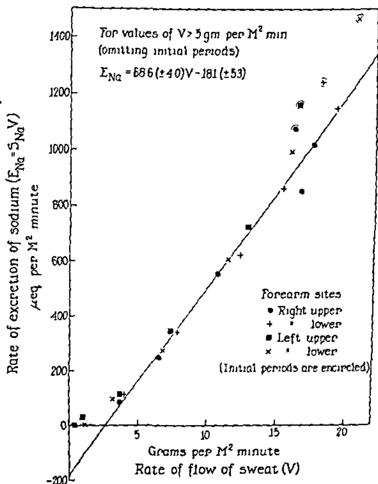


Figure 8. Relation of rate of excretion of sodium in sweat and rate of flow of sweat. Reprinted from The Journal of Clinical Investigation, 35:114, 1956.

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Water reabsorption

If urea concentration is a measure of water reabsorption by the sweat gland, the urea concentrations in the sweat should indicate whether there is greater or less water reabsorption by the ducts of patients with cystic fibrosis. The mean concentrations of urea in the sweat of the controls and of patients with cystic fibrosis of the pancreas were found to be essentially identical. The concentrations of urea in the plasma were also the same in the two groups, as were the ratios concentrations of urea in sweat to that in plasma (table 6).

Table 6

Ratio of urea in sweat to urea in plasma

Controls	$\frac{58.5 \text{ mg/100 ml}}{32.8 \text{ mg/100 ml}} = 1.78$
Patients with cystic fibrosis	$\frac{55.84 \text{ mg/100 ml}}{30.8 \text{ mg/100 ml}} = 1.81$

Sodium reabsorption

Concerning the possibility that there is diminished sodium reabsorption in the sweat glands of patients with cystic fibrosis, it seems unlikely there would be a specific instance in which reabsorption of sodium was diminished, since in other states where sodium concentration in the sweat is high, there may actually be greater sodium reabsorption, as shown by the regression equations (figure 8). Furthermore, any system based on reabsorption of sodium cannot be generalized to involve other glands, which is one of the prerequisites for solution of the problem of cystic fibrosis.

Sodium concentration in the precursor solution

By use of standard thermodynamic equations of the form $\Delta G = nRT \ln \frac{\text{Plasma concentration}}{\text{Sweat concentration}}$, a curve similar to that in figure 9 is evolved. When the sodium concentration rises in the sweat, there is actually an increased gain for the gland; ΔG is positive. Above a maximum of 50 to 55 mEq Na/l of sweat, ΔG decreases. With a continued increase of sodium in the sweat, the free energy gain for the gland decreases until there is no free energy gain for the gland itself.

In calculating ΔG, the total free energy change, for sweat of varying sodium concentrations, the relationship shown in figure 10 obtains. This is the ΔG for transport of all the materials comprising the glandular secretion. As can be seen, the minimum free energy change, *the point at which minimum work is required for secretion,* is in the vicinity of a concentration of sodium in sweat of 90 to 100 mEq/l. Most of the work is due to the transport of water. The sweat of patients with cystic fibrosis is secreted with considerably less expenditure of energy than that of the individual whose sweat has a normal concentration of sodium These same calculations can be applied to the secretion of all glands.

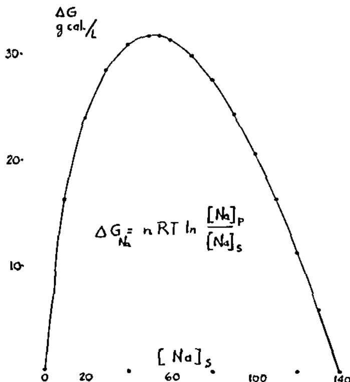


Figure 9. Free energy change (ΔG) for sodium in sweat when secreted at various concentrations.

If speculation is permissible, it might be interesting to consider the concept of some common denominator affecting all glands responding to cholinergic stimuli, that common denominator being a

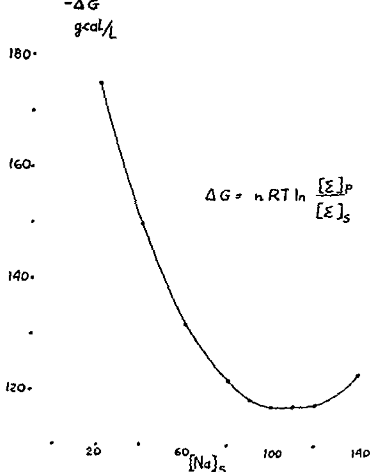


Figure 10. Total free energy change (ΔG) for the sweat gland at various concentrations of sodium on the sweat.

shortage of energy for secretory activity In the presence of a diminished energy supply, secretions will be formed that require a relatively low free energy change When less energy is available, two things will occur, a rise in concentration of solid materials and, if the load is great enough and concentration has attained an optimal level, a *limitation of rate of secretion* Such findings have been obtained when arterial occlusion was carried out

What chemical systems would be involved in such a deficit of energy are unknown, and considerably more work must be done on the energetics of secretion to confirm or deny this hypothesis.

Studies of Sweat in Fibrocystic Disease of the Pancreas

Clinical findings

DR. PAUL A. DI SANT'AGNESE: In 1952 Kessler and Andersen reported that 5 of 10 patients hospitalized during a severe hot spell in August, 1948, were patients with fibrocystic disease of the pancreas They pointed out that such patients responded adversely to periods of high atmospheric temperature.

Since there was already some evidence that in fibrocystic disease there may be a disturbance of multiple glandular structures, and since the sweat glands enter into the physiologic picture in a major way only during hot weather, the objectives of original studies were two, to measure the amount and electrolyte composition of sweat in patients with this condition, and to attempt to relate the findings to clinical casualties and to the pathologic physiology of the disease

The group of patients studied has now been increased to 90 with fibrocystic disease and to 151 controls, of which 135 are children 6 weeks to 16 years, and 16 are adults, 35 to 62 years of age. A description of the technique for collection has been published

Seventy-nine of the patients presented the classical clinical and laboratory features of fibrocystic disease In all these, one or more duodenal assays have shown the absence of pancreatic enzymes Eighteen have died subsequent to the studies. Autopsies of 14 showed the pathologic changes typical of the disease. In the remaining 11 patients, tryptic activity was still present in duodenal contents, however, the disease was strongly suspected on clinical grounds. Two of these died subsequent to the sweat studies, and autopsy in both has confirmed the diagnosis

Specifically excluded from the control group, which included patients with a variety of conditions, were those with known adrenal insufficiency or renal disease and members of the families of patients with known fibrocystic disease

The range of concentration of chloride was 60 to 160 mEq/l in the patients with cystic fibrosis of the pancreas, with a mean of 106, and in the controls 4 to 60 mEq/l, with a mean of 32 (figure 11). The range of values for concentration of sodium in the sweat of the patients with fibrocystic disease was 80 to 190 mEq/l, with a mean of 133, and in the control subjects, 10 to 90 mEq/l, with a

mean of 59. Age or color did not seem to make any difference in the concentrations of electrolytes in sweat.

The mean volume of sweat was not significantly different between the patients with fibrocystic disease and the control group of patients

An effect of pancreatic deficiency or of chronic pulmonary disease was excluded by the finding of the same concentration of

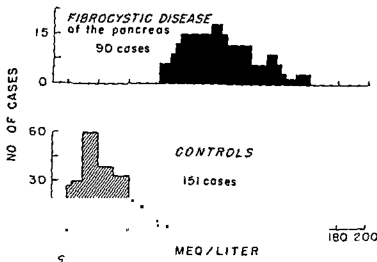


Figure 11. Concentration of chloride in the sweat of patients with fibrocystic disease and of a control group Sweat is from midabdomen or scapular region Reprinted from The Journal of the American Medical Association, *160* 846, 1956

electrolytes in sweat in patients with fibrocystic disease with or without pancreatic insufficiency or respiratory disease In addition, two groups of patients adults with acquired pancreatic deficiency and adults and children with a variety of chronic pulmonary diseases of other origin, were found to have normal concentrations of electrolytes in the sweat The effects of the administration of pancreatin or antibiotics were also eliminated by similar methods.

Data on a few patients with fibrocystic disease has been published showing a normal adrenal response to ACTH stimulation, a normal ability of the kidney to conserve base and a slight decrease in sweat electrolytes on administration of DOCA. These findings constitute evidence that impaired adrenal and renal function cannot

be significant factors in the production of the abnormal concentrations of electrolytes.

Thirty-one patients of the group with fibrocystic disease had normal concentrations of sodium in the serum; 10 patients with respiratory acidosis, either uncompensated or compensated, had normal concentrations of sodium in the serum.

Six patients with cystic fibrosis of the pancreas, in acute dehydration during hot weather, had low concentrations of sodium and chloride in the serum. The concentrations of potassium were not altered, nor was pH.

Heat appe
which results
abnormal con
is a so-called pure salt depletion in which primarily the extracellular fluid compartment is affected. The circulatory changes produced by salt depletion are much more severe than those in so-called pure water depletion, and produce an effect similar to the shock that follows extensive burns, trauma or hemorrhage.

These studies suggest that acute loss of salt through the sweat should be suspected when patients with fibrocystic disease, with vomiting and dehydration, are seen during a period of hot weather Thirst is an unreliable symptom Hyperpyrexia, cardiovascular collapse, and, at times, coma represent further steps in salt depletion and bring about an acute medical emergency. The patient may be in imminent danger of death unless prompt and vigorous measures are undertaken to restore the depleted water and electrolyte stores. Physiologic saline is the first urgently needed solution; other repair solutions should be administered as indicated.

Sweat in other conditions

Some of the conditions in which results of the sweat test have been normal are shown in table 7

Sixty members of families of patients with known fibrocystic disease have been studied. Fourteen had abnormally high electrolytes in the sweat and of these, six had unusual susceptibility to long-lasting and severe respiratory infections In three of these, varying degrees of generalized obstructive emphysema and chronic bronchopneumonia were demonstrated roentgenographically It was possible to test all members of 15 families In the others, one or more individuals were missing. In two of the 15, both parents were affected; in three others, one of the parents and, in the remaining 10, electrolytes were normal in both parents

Electrolytes in other body fluids

The data in table 8 come from analysis of electrolytes in mixed saliva There is a statistically significant difference in the

concentration of sodium and chloride between the patients with cystic fibrosis of the pancreas and the control group, which represent a variety of other conditions. This is not true of concentration of potassium.

Table 7

Some conditions with normal concentrations of electrolytes in the sweat

Chronic Pulmonary Disease
- a. Bronchial asthma
- b. Familial dysautonomia
- c. Agammaglobulinemia
- d. Miscellaneous

Idiopathic Celiac Disease
Cirrhosis of the Liver
Acquired Pancreatic Deficiency

Table 8

Electrolytes in mixed saliva (mEq/L)

		Cl	Na	K
Cystic Fibrosis of Pancreas	Mean	24	27	21
	S D	(± 6.1)	(±10.7)	(± 6.1)
Controls	Mean	16	16	20
	S D	(± 4.9)	(±10.6)	(± 5.3)
Difference (Δ M)		8	11	1
S E.Δ M.		1.97	3.8	2.1
Δ M / S E Δ M		4.1	2.9	0.48
Probability (P)		< 0.01	< 0.01	—
Number of Cases	C F P	16	16	16
	Controls	15	15	15

Studies of the parotid secretory rate done with a suction cup and graduated pipet reveal that patients with fibrocystic disease have a significantly increased parotid secretory rate as compared to the control group (table 9).

Investigated with negative results were mixed salivary amylase, lysozyme and electrolytes in parotid saliva. The viscosity of mixed and parotid saliva was found to be slightly less than that of the control.

Since most patients with fibrocystic disease have chronic lung disease and continually swallow mucous, it was difficult to know whether the electrolytes in the stomach come from the pulmonary or gastric secretions. The electrolytes in the duodenal contents were investigated and found to be quite normal

Excretion of chloride or sodium in the stool, measured during balance studies, was not altered

Table 9

Parotid secretory rate

		(ml/5 min)
Cystic fibrosis of pancreas (16)	Mean	0.30
	S D	± 0.194
Control group (23)	Mean	0.125
	S D	± 0.10
Difference (Δ M)		0.175
S E Δ M		0.052
$\frac{\Delta M}{SE \Delta M}$		3.36
Probability (P)		< 0.01
Number of cases	C F P	16
	Controls	23

Summary

In cystic fibrosis of the pancreas there is a specific electrolyte abnormality of sweat. The concentrations of chloride and sodium in the sweat are markedly increased in patients with fibrocystic disease, as compared with normal individuals or patients with a variety of diseases In contrast, the volume of sweat is not different in the two groups Failure of the sweat glands to conserve salt clarified the unusual susceptibility of patients with fibrocystic disease to heat, through acute loss of salt in the sweat It also offered, from the practical standpoint, a means of treating heat casualties and of preventing their occurrence by the administration of additional salt to the diet.

In all cases showing the characteristic anatomical changes of fibrocystic disease at autopsy, sweat tests (when performed) had shown an abnormality in the concentration of electrolytes. In about

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of determinations has not been great between patients and controls, and I wonder whether this could not be considered a serviceable clinical test for smaller institutions

DR. LOWE: Di Sant'Agnese has published some balance data on administration of DOCA for short periods of time to patients with fibrocystic disease. I would interpret the data as showing some change in the concentration of electrolytes in sweat in response to DOCA. I wonder if this might be an abnormal response

Table 10

Sweat electrolytes

	Sodium mEq/l		Chloride mEq/l	
	tube	gauze	tube	gauze
Fibrocystic patients (12)				
No of determinations	12	9	9	8
Range	102-198	110 6-215 4	103 1-216.9	116 220
Mean	161 5	162 4	156 8	167 3
Control group (40)				
No of determinations	40	31	39	31
Range	1 1-101 5	1 0 88 2	9.3-111 8	8 0 93 0
Mean	31 9	25 8	42 5	36

DR. DI SANT'AGNESE: There is some response, but the response is not very great, and the concentrations of electrolytes are still in the very high range But with each one of these factors, salt restriction, heat or DOCA, there was some decrease in the concentration of electrolytes in sweat.

DR COOKE: I think the response of the sweat gland to DOCA is of some interest. In continual administration of DOCA to a normal individual, there is a striking reduction in the excretion of sodium and chloride via the kidney for two or three days This excretion returns to normal levels, but the electrolyte concentration in the sweat remains at a very low level, indicating an entirely different response from that of the kidney tubule

DR BESSMAN If there is a lesion in the generation of energy, it would be rather difficult to understand how the sodium of the sweat, even if there were some evaporation, is still greater than that of plasma

DR. DI SANT'AGNESE I should like to point out that the determinations in which the concentration of sodium and chloride in the serum remain unexplained We have thought there might be a technical error, due to some cause such as evaporation

99 percent of living patients with the typical pulmonary and pancreatic findings, electrolyte abnormalities also existed in the sweat. Patients with adrenal insufficiency have not been tested.

The diagnosis has been limited, up to the present, to that fully *manifested disease in patients with complete pancreatic deficiency*, respiratory disease and abnormal sweat electrolytes. However, gradation in or absence of involvement of the pancreas, lungs, liver and, perhaps, the sweat glands is inherent in this condition. Of course, it is possible that in every instance all susceptible organs are affected. but perhaps sometimes to an extent too limited to give rise to clinical manifestations.

It must be concluded that the generalized disease called fibrocystic disease of the pancreas may occur in patients in whom pancreatic function is normal, and that the presence of tryptic activity in the duodenal juice in normal concentration does not negate the diagnosis.

The mixed salivary and the parotid secretion studies indicate that there are three different defects in exocrine secretions that need to be explained a probable abnormality of mucous secretion giving a reasonable explanation for the pancreatic, hepatic and possibly the pulmonary symptoms; an abnormal concentration of electrolytes in eccrine sweat and mixed saliva, and, finally, an increased parotid secretory rate. Therefore, these exocrine glands, different in function and in the products they elaborate, are affected in different ways. *It would seem that dysfunction of the autonomic nervous system* may be the common denominator responsible for this widespread disturbance.

Discussion

Dr. Barbero. We have attempted to find a simple method for the collection of sweat which will give results comparable to the Darling technique of patch collection.

An infant less than six months of age, previously washed, is placed in an Isolette®, on a plastic sheet With the Isolette® set at 90° F and 50 percent humidity, in a period of 5 to 25 minutes the sweat that has collected on the plastic sheet can be aspirated with a syringe. With an older child, a heat cradle and a 100-watt bulb are placed over the trunk. It is obvious that such a gross technique has a serious potential error of evaporation.

Table 10 compares the values obtained from patients with fibrocystic disease and a control group by the gauze patch technique of Darling and by what I have called the tube method. The overlap

Diagnosis of Fibrocystic Disease of the Pancreas and Partial Pancreatic Insufficiency

DR. HARRY SHWACHMAN: In the past the diagnosis of fibrocystic disease of the pancreas has been based largely on tests of pancreatic function. More recently it has become obvious that a few of the children who succumb with this disease have demonstrated none of the changes in pancreatic function during life that we consider characteristic of the disease This, of course, immediately throws doubt on the validity of the laboratory examination of pancreatic function in making the diagnosis Among complicating factors are the adequacy of the duodenal fluid specimen, and the accuracy of the laboratory determination itself.

Table 11 lists some of the duodenal fluid measurements They are divided into those which provide a direct measurement of pancreatic function and those which are indirect measures of pancreatic function

In addition to the reduced volume of duodenal secretion that occurs in pancreatic insufficiency, there may be increased viscosity and change in pH. If there is complete pancreatic failure, the stimulation of the pancreas by intravenous secretin or by intraduodenal instillation of olive oil no longer seems to have effect.

The enzyme determinations in the fasting state do not necessarily reflect what reactions the various tests will show following stimulation If there is diminished fasting activity on repeated occasions, it may still be possible to demonstrate a rise in enzyme activity following the stimulation I think this has important implications in attempting to evaluate balance studies on patients with this disease who, in their fasting state, show no activity, yet, after stimulation with food or with olive oil or secretin, produce some measurable activity.

In reporting the enzyme activity or other measurements of duodenal fluid, it is important to describe exactly what is being measured Our experience has shown that the level of activity of a single enzyme may not be representative of others present in duodenal fluid,

DR. COOKE: Studies on palmar sweat show that intermittent sweating gives concentrations of electrolyte that are several times the concentrations when the sweating is profuse; these concentrations may be of the order of twice the isotonic concentration of sodium. Urea concentrations are also tremendously elevated, about 1,000 mg/100 ml.

When there is profuse sweating there is a constant flow, with minimal opportunity for reabsorption of water back through the duct or into the skin or wherever else it goes. The urea concentration is useful as a control for evaluation of electrolyte concentrations Schwartz finds the ratio of the concentrations of sweat urea to plasma urea is very close to 1.75, despite extreme variations in levels of urea in plasma.

In order to draw the conclusion that the sweat is hypertonic, we would have to be certain there is not a very high urea concentration in that same sweat, to ensure that there had been no back diffusion or evaporation.

DR. ZACHARIAS DISCHE: Has anyone determined the lactic acid content of the secretions from patients with cystic fibrosis? *If there is a defect of the energy transfer in this diseased tissue, we might expect a decrease in lactic acid formation during secretion This may be a very easy way of getting some conclusive indications of the mechanisms involved*

DR. COOKE: We are in the process of determining concentrations of lactic acid. *I avoided the lactic acid problem in my discussion because, at the moment, it seems to be in a confused state* with regard to concentrations in sweat Investigators who have attempted calculations feel that there is enough lactic acid in a liter of sweat to account for the energy requirement for a dilute sweat. What the actual source of the lactic acid is, whether the plasma or the metabolism of the gland, remains to be determined

DR. DI SANT'AGNESE: May I ask Cooke *to comment on the solutions to be used in patients who have acute salt loss?*

DR. COOKE: *I should think expansion of extracellular volume* initially with something resembling extracellular fluid, namely saline, would be the obvious first step Certainly, it is worthwhile to expand volume initially because the hyponatremic patient may have very marked elevation of the serum potassium, even though he is short of potassium, and he is particularly susceptible to potassium intoxication. Replacement with a potassium containing solution is the next step.

If there are symptoms or signs of water intoxication, which I do not believe your patients exhibited, a more rapid elevation of sodium concentration with the use of three percent sodium chloride ... before the initial volume expansion

abnormal values obtained by the usual measures of pancreatic function Table 12 shows the ranges that we have found in the various measurements. It would seem that even the roughest test for pancreatic function would, perhaps, be useful, even though it did not necessarily indicate intermediate ranges.

Table 12

Duodenal fluid findings

Test	Healthy infants and children	Celiac disease	Complete pancreatic insufficiency	Partial pancreatic insufficiency
Volume, ml/hr	8-25	20-45	1-10	5-25
pH	6 0 8.4	6 0-8 4	3 5-8.1	5 0 8 4
Bile	20-125	15 75	10-125	10 125
Viscosity time, min	below 3	below 3	over 3 in 87 percent patients	over 3 in approximately 70 percent patients
Amylase, units	4 20	4 20	0 1	0 20
under 6 mos	0	0	0	0
Lipase, units	3-10	3 10	0-1	0 10
Protease, units	18-70	18 70	0-2 5	0-70
Carboxypeptidase, mg β-naphthol*	0 40 1 00	40-1 00	0-0 40	0 00-1 00
Chymotrypsin mg β-naphthol*	11 65	11 65	0-5	0 65

**Based on examination of approximately 250 patients The other values are based on a study of over 1,000 patients The numbers refer to range of activity of the enzymes as defined and determined in our laboratory*

The viscosity test can be carried out in about 90 percent of the patients examined It cannot be done on all because of the technical difficulties We do not centrifuge or filter the specimen but measure it, using an Ostwald viscometer immediately after collection In most patients with compete pancreatic insufficiency, the viscosity time is well over three minutes

Since amylase is absent under six months of age in all children, there is no need for the determination of the amylase in the diagnosis of disease in small infants The method used for this determination is the standard procedure described by Free and Meyers.

Indirect measurements, such as nitrogen and fat balances are useful. These are probably not of great importance in diagnostic work, but are of fundamental importance in an understanding of the disease.

All the diagnostic methods that have been devised are not listed here. For example, the antitrypsin titrations reported by the group in England we found of no great help. Measurement of excretion of iodine in the urine after administration of Lipiodol ® by mouth has not proven reliable as a diagnostic procedure.

Table II

Laboratory tests for pancreatic function

Direct	Indirect
Duodenal Fluid Volume — Before and after stimulation *Bicarbonate* or pH — *Before and after* stimulation Viscosity Enzyme activity (amylase, lipase, proteases) (1) Fasting state (2) After stimulation (a) intraduodenal olive oil (b) intravenous secretin	*Metabolic balance* Nitrogen and fat intake & output
	Stool examination (1) Gross examination (see Metabolic balance) Excess bulk with increase in total fat and nitrogen (2) Microscopic examination Fat Starch Proteins (Schmidt test) (3) Enzymes—trypsin
	Absorption tests (1) Fat (a) cream chylomicron counts fatty acids (b) vitamin A esters vs alcohol and aldehyde serum levels of vitamin A (2) Proteins (a) gelatin or casein absorption tests (b) I^{131} labelled casein
	Clinical response (1) To dietary adjustment (2) To an adequate potent pancreatin preparation

Clinical response appears at the end of this table because this may give a clue as to whether pancreatic insufficiency exists or not This is certainly not a very scientific way of demonstrating anything, but I think that when there is doubt we might get some help by this technique

There has been considerable discussion about the tremendous range of activity between the average normal and the definitely

with normal sweat electrolytes. Have the latter children been repeatedly tested to be sure these values were correct?

DR. SHWACHMAN: We examined 90 parents, and found about 21 to have abnormal concentrations of electrolytes in the sweat. We found two pairs of parents where both father and mother had abnormalities. Repeat sweat tests have been performed in the atypical cases.

DR DI SANT'AGNESE. In our studies, 100 percent of patients who have died and have shown pancreatic change at autopsy have had an abnormal sweat. At Babies Hospital we have not seen patients with cystic fibrosis who did not have abnormal sweat; however, I feel sure that a patient with cystic fibrosis may have normal sweat electrolytes

DR ZUELZER: How much variation within the same individual is observed over a period of time? Is there any progression of the sweat anomaly comparable to that in the enzyme anomalies in the duodenal juice?

DR DI SANT'AGNESE. While there was some variation in repeated testing of one individual a few weeks or a few months apart, there has been no evidence so far that time or increase in age makes a difference in electrolyte concentration

DR. ROBERT G FRAZIER In trying to set up some of our diagnostic methods for the assay of enzymes in pancreatic juice, we studied the gelatin viscosity determination as described by Leubner and Shwachman We found that the accuracy of the method was markedly decreased by small variations in the ratio of initial to final viscosity

When the proteolytic activity was carefully determined by both the gelatin viscosity method and the Andersen-Early method simultaneously, the results were comparable It is my feeling that the Andersen-Early modification of the Fermi method is simple to perform, reliable in its ability to measure proteolytic activity, and requires no special equipment The viscosimetric method has the potential for greater accuracy, but this is difficult to achieve

DR J ALBRIGHT JONES I would like to report some data obtained from duodenal intubations Material aspirated from the duodenum during a routine intubation may consist of a mixture of duodenal and gastric juices A study was made to determine whether it is important to exclude gastric contents from the duodenum during the collection of duodenal juice for enzymatic analysis

Sixty-three patients, none of whom had cystic fibrosis of the pancreas, were selected A bilumen Miller-Abbott tube, with the openings so located that gastric and duodenal juices could be separately aspirated, was used A sample of duodenal juice was collected by routine intubation Then the stomach was emptied as completely

The method for lipase utilizes olive oil. The protease method employs casein as a substrate, and the liberated tyrosine is measured.

Carboxypeptidase and chymotrypsin are two enzymes of interest because they have not been measured regularly in patients with pancreatic insufficiency. Determination of the former is not now one of our routine measurements, but we measured it in a series of patients and found that in complete pancreatic insufficiency this enzyme is deficient, just as is protease. Chymotrypsin is of interest because it will attack certain proteins, including casein, much more effectively than trypsin. Chymotrypsin attacks gelatin less efficiently than trypsin

The standard procedure used routinely in our laboratory for determination of proteolytic activity is the Andersen-Early modification of the Fermi method. We also use a method based on the change in viscosity of a gelatin substrate in the presence of duodenal fluid. In this method the amount of enzyme present can be calculated from the change in viscosity in the one-hour incubation period. We feel that this test is a more sensitive measure of proteolytic activity than is the Andersen-Early test, in which the end point depends on liquefaction of well over 80 percent of the substrate.

We studied a fairly large number of children with all types of conditions and have not encountered any condition in which the concentration of electrolytes in the sweat is as high as it is in this disease. We found three or four patients with fibrocystic disease in whom the concentration of electrolytes in the sweat was in the normal range.

I should like to emphasize the great importance of taking very careful and accurate histories and, also, of having the faculty of doubting certain statements that are made by parents who are well-intentioned but who, perhaps do not have the experience necessary to report accurately on observations concerning the development of their infant.

Discussion

Dr Stephen W. Royce I should like to know whether Shwachman has any figures on abnormalities in the sweat of the parents of his child patients I think such data might be used to set up a screening test for parents

Di Sant'Agnese cited abnormalities of sweat electrolytes in 100 percent of patients, while Shwachman stated he found three or four

The Chemistry of Mucus

Dr Zacharias Dische Clues to the understanding of the chemistry of mucus came from a knowledge of the chemistry of related compounds. These are primarily the so-called blood group substances, which were discovered at the beginning of the twentieth century and were characterized initially on the basis of their serologic behavior.

It was soon found that the gastric juice, mucosa of the stomach and other mucoid substances from the intestines displayed some features, including the specificity, of the serologic behavior of blood group substances Urine and other substrates, such as saliva, were also found to contain these compounds

In 1946 it was found that the blood group substances obtained from the mucosa of the stomach of the pig contained a sugar that up to this time had not been found in animal tissue. This was so-called fucose, a methylpentose or 6-desoxy-hexose. About one or two years later we succeeded in developing a sensitive and simple method of detecting and determining this type of sugar.

Mucus of the respiratory tract and all kinds of mucus of the intestinal tract and of the urogenital tract were found to contain compounds similar in composition to blood group substances consisting of galactose, hexosamine, later found to be a mixture of two different kinds of hexosamine, and fucose Quantitative determination indicated that the amount of fucose is very similar in all these compounds In human mucus, the amount of fucose varies between 13 and 18 percent of the total sugar The ratio between the galactose and fucose moieties is between two and three to one; for

hexosamine, and fucose These are combined in such a way that the aldehyde group of carbon 1 of one sugar is attached to one of the hydroxyl groups of another sugar, forming long chains of repeating units There is convincing evidence that fucose is attached to the hydroxyl of another sugar so that its hydroxyl groups are all free

Mucopolysaccharides of this type are also associated with serum proteins, particularly globulins, and are present in almost all animal tissues Without attempt at strict definition, this combination of

as possible, and kept emptied by frequent aspiration during the collection of a second duodenal specimen Determination of pH and enzymatic analyses for trypsin, amylase and lipase were made on the two samples; comparison showed no important or consistent differences between the two specimens. It is concluded that the customary method of collecting one duodenal specimen for enzymatic analysis is satisfactory.

DR. MAY: At what age does the anomaly of secretion of sweat appear? Have measurements been made at birth?

DR. SHWACHMAN: We have been able to measure sweat in infants between four and five weeks of age, and have found the defect present. We have not been able to get enough sweat from the smaller infant.

DR DI SANT'AGNESE: We have recently examined a number of children about six weeks of age with the same results.

DR BARBERO: We have studied two infants with meconium ileus shortly after the convalescent period, at 7 to 10 days of age, and these have shown abnormal sweat electrolyte concentrations. However, the production of sweat in these infants appeared to be the exception to the rule, since we have had no success in collecting sweat generally before two to three months of age

DR. SHWACHMAN: It has been shown that meconium from patients with pancreatic fibrosis is abnormal in its chemical composition Our observations have confirmed the presence of protein, which is abnormal Normal meconium will not precipitate when protein precipitating agents are added, the abnormal meconium in these patients will This can be of practical value at the time of operation To determine whether the condition is atresia or meconium ileus, a 20 percent trichloroacetic acid solution can be utilized as a precipitating agent to test the meconium.

A similar analysis of mucus from patients with cystic fibrosis is shown in table 14. In the ratios of fucose to galactose and of hexosamine to galactose, there is no fundamental difference as compared with the normal fluid except, perhaps, in one instance where the fucose is low.

Table 13

Amount and composition of the mucoid fraction of the duodenal fluid of normal individuals

All values in mg/100 ml of duodenal fluid

Specimen	Protein	Glucose	Galactose	Fucose	Hexosamine	Mucopolysaccharide % of mucus
I		19.0	47.0	39.5	54.5	
II	218	7.0	31.0	29.0	44.2	0.30
III	217	11.5	19.8	21.0		
IV		6.7	28.8	18.5		
V	265	2.0	21.2	10.7		
VI		13.0	13.5	13.5	17.7	

Table 14

Amount and composition of the mucoid fraction of the duodenal fluid from patients with cystic fibrosis of the pancreas

All values in mg/100 ml of duodenal fluid

Specimen	Protein	Glucose	Galactose	Fucose	Hexosamine	Mucopolysaccharide % of mucus
I		15.6	8.3	6.6		
II	291	9.0	30.0	32.0	37.7	0.28
III	662	12.5	24.5	4.5		
IV		13.0	11.2	15.0		
V	228	2.5	14.7	10.0		

The ratios between fucose and galactose and between hexosamine and galactose in both groups, with one exception, show no significant difference. This one very simple measure so impressive as a differentiating criterion with blood group substances, showed

mucopolysaccharide and protein has been called a mucoprotein or, if the content of the total unit in carbohydrate is low, glycoprotein

Mucoproteins are not so much characterized by the amount of carbohydrate but by the fact they do not have the properties we expect of the more familiar proteins. For example, they are very often not precipitable by hydrochloric acid, nor by trichloroacetic acid, which is one of the most powerful and complete precipitants. The mucoproteins can be classified by the nature of the mucopolysaccharide.

Apparently, fucose is able to condition the surface of the polysaccharide and, in a combination of polysaccharide with the protein, to condition the surface of the protein. This is essentially the same role played by the residues of amino acids in the protein molecule. The fact that the content of fucose varies to a great degree within various types of mucus in a specific species, between individuals of the same species and between species bears out this supposition.

Analyses of mucus in duodenal fluid

In our studies, we found the content of fucose in mucoids from serum may vary, in the different mucoproteins and in different individuals, particularly in disease, up to 200 or 300 percent. It is a mobile component that may be of major importance.

It seemed possible that the change in viscosity of the duodenal fluid in patients with cystic fibrosis could be due to a difference in the degree of polymerization of the mucopolysaccharides, which might be associated with differences in the ratios of various sugars. This might offer the opportunity to differentiate it from the duodenal fluid of normal children

The result of the analysis of the intestinal fluid of normal individuals is shown in table 13. The mucoprotein fraction of the fluid contains fucose, hexosamine, galactose and one or two other hexoses calculated as glucose There is almost no relation between the amount of "glucose" and the amount of galactose The "glucose" in the duodenal fluid comes from other sources than the mucoproteins.

There is an equivalence between fucose and galactose, except in one instance where the difference is beyond the limit of error of the method.

The lack of a stoichiometric relation of hexosamine and galactose can probably be explained by the presence of another type of polysaccharide found in all forms of mucin This is sialic acid, which is a hydroxy-keto acid of known structure. It is apparently *combined* with hexosamine, and this excess of hexosamine over galactose may be due to the inclusion of sialic acid.

Histochemistry of Mucus and Exocrine Glands

DR. BENJAMIN H. LANDING: Some but perhaps not all mucus-secreting glands of endodermal origin are affected in cystic fibrosis of the pancreas, and all susceptible glands may not be affected in all patients. Whether mucus glands arising from other germ layers can be affected depends on the outcome of as yet unresolved disputes about the embryologic and functional positions of the salivary glands and on studies on the genitourinary tract glands. I do not think we can say whether the pattern of involvement is conditioned by the fact that those glands most clearly affected are structures with ducts which would be more easily obstructd mechanically

We decided to confine our studies to airway, pancreas, biliary tree, stomach, small intestine, and colon, where the specific defect involves mucus, utilizing selected blocks of these tissues, from a group of patients of varying age with fibrocystic disease and a group of control patients All the tissues were fixed in cold acetone and embedded in paraffin, for the reason that this is a method which allows study of some enzymes as well as the mucin. We used 40 blocks in all, and carried sections of each block through a total of 40 procedures. We chose to devote most attention to mucopolysaccharides, chiefly because histochemical methods for the mucopolysaccharides are much better developed than are those for proteins.

The classical histochemical procedure for the demonstration of the carbohydrate moieties of mucoproteins is the periodic acid leukofuchsin, or PAS procedure Periodic acid oxidizes the carbohydrates to carbonyl groups which can bind leukofuchsin as a red dye For demonstrating acidic groups, such as the groups of carboxylic acids like mucic and glucuronic acids, or ester sulfate, we used a basic thiazine dye, toluidine blue (TB).

Aldehyde-fuchsin (AF) stains both acid groups and mucin generally The mechanism of the more general mucin-staining power is not known, but there is some evidence the triphenylmethane dyes as a class have an affinity for mucins

Results of tissue stains

As shown in table 15, there are no significant differences which we can recognize between the staining powers of mucus from normal

no differences between the mucopolysaccharides from normal individuals and from patients with cystic fibrosis.

Discussion

DR. GIBBS: We have determined the serum β-glucuronidase in normal infants and children (25) and in patients with fibrocystic disease of the pancreas (15). There is an elevation of borderline statistical significance in the serum levels of children *with fibrocystic* disease; this is probably explained by the pulmonary infection

We have also determined the serum mucoproteins in the two groups by the method of Winzler. There was no difference found by this method.

DR. BESSMAN We have made a somewhat similar investigation of the salivary mucoprotein We measured hexosamine, acetylhexosamine and sialic acid in several patients with cystic fibrosis and in a number of normal children There was no distinguishing feature between the two groups.

DR BARBERO We have investigated the protein fraction of duodenal fluid in controls and in patients with fibrocystic disease After dialysis and concentration of duodenal fluid, the proteins have been separated by paper electrophoresis At a pH of 8 5 we have obtained a band moving in the opposite direction from that in controls This could be a different protein or a difference in polymerization. We have not tested saliva.

patients and patients with cystic fibrosis, with regard to PAS, toluidine blue or aldehyde fuchsin staining. The last three columns show the effect of a variety of oxidizing agents. Sodium bismuthate and lead tetra-acetate are two reagents which produce approximately the same results as periodic acid.

... that will con- ... which can be ... th this disease may show a greater tendency to take this stain, but this is the only finding suggesting difference. This may indicate some link in the mucus of patients with cystic fibrosis which is more easily ruptured by sulfuric acid-permanganate.

Table 17

Toluidine blue binding at various pH

	2	3	4	5	6	7
Controls						
Pancreatic ducts	–	–	–	±	–	±
Stomach	–	–	–	–	–	–
Small intestine	–	±	±	±	±	±
Brunner's glands	–	–	–	–	–	–
Colon	±	±	+	+	±	+
Larynx	±	±	±	±	±	±
Bile ducts	–	–	–	±	±	±
Pancreatic fibrosis						
Pancreatic ducts	± to –	± to –	± to –	±	±	± to –
Stomach	–	–	–	–	–	± to –
Small intestine	–	± to –	±	±	±	+ to ±
Brunner's glands	–	–	–	–	–	–
Colon	±	+ to ±	+ to ±	+	++ to +	++ to +
Larynx	±	±	±	+ to ±	+ to ±	+ to ±
Bronchioles	–	–	–	±	–	–
Bile ducts	–	–	± to –	± to –	± to –	±, –
Gallbladder	–	–	±	±	±	±

Table 16 gives the findings of the preliminary attempts to study the solubility of these mucuses, simply by extracting them with water for 24 hours at varying pH. There are no obvious differences.

Table 17 shows the results of staining with toluidine blue in solutions at various *pH* values, more strongly acidic groups bind

Table 15

Basic procedures and effects of oxidants

	PAS	TB	AF	Bi S	Pb S	K Mn O₄ AF
Controls						
Pancreatic ducts	+	± to —	—	±	±	—
Stomach	++	—	—	++	++	—
Small intestine	++	±	±	+	++	±
Brunner's glands	++	—	—	++	+++	—
Colon	±	+	+	±	±	++
Larynx	++	±	±	++	+	±
Bile ducts	±	±	—	±	±	±
Pancreatic fibrosis						
Pancreatic ducts	++	± to —	± to —	++	++	± to —
Stomach	++	—	—	++	++	±
Small intestine	++	±	±	++	++	±
Brunner's glands	++	—	—	++	+++	± to –
Colon	±	+	+	±	+ to ±	++
Larynx	++	±	+	++	++	++ to +
Bronchioles	±	—	—	—	—	—
Bile ducts	+ to ±	± to —	± to —	±	+ to —	±
Gallbladder	±	+ to ±	—	—	±	±

Table 16

Extraction at different pH—PAS *staining*

	2.5	4.5	6.5	8.5
Controls				
Pancreatic ducts	+	+	+	+
Stomach	++	++	++	++
Small intestine	+	+	+	+
Brunner's glands	++	++	+++	++
Colon	±	±	±	±
Larynx	+	+	+	+
Bile ducts	±	±	±	±
Pancreatic fibrosis				
Pancreatic ducts	++	++	++	++
Stomach	++	++	++	++
Small intestine	+	+	++, +	++, +
Brunner's glands	++	++	++	++
Colon	+, ±	+	+	+
Larynx	++, +	++, +	++	++
Bronchioles			-	±
Bile ducts	+	+	+	+
Gallbladder	+, ±	+, ±	+, ±	+, ±

toluidine blue at lower pH Again, there is a rather questionable increase in toluidine blue binding generally in the mucus of the patients with cystic fibrosis It is more apparent at higher pH, suggesting that these mucins have a higher content of a weakly acidic group, possibly carboxyl, as opposed to strongly acid sulfate ester. I do not know of any phosphate ester groups normally occurring in the mucins, except for glycogen.

Table 18 shows results from tests with pepsin, trypsin, hyaluronidase, and streptokinase plus streptodornase (SKD). There may be slightly greater resistance to digestion with trypsin of the mucins of patients with fibrocystic disease.

Table 19

Miscellaneous determination

	Lipase	SH	SS	Acetylation-PAS
Controls				
Pancreatic cells	+	+	±	—
Pancreatic ducts	—	±		—
Stomach	—	+		±
Small intestine	—	+	±	±
Brunner's glands	—	±	±	±
Colon	±	+	—	—
Larynx	—	+, ±	—	±
Bile ducts	—	±		
Pancreatic fibrosis				
Pancreatic ducts	+, —	+, ±	+, ±	±, —
Stomach	—	+	±	+, ±
Small intestine	—	+	±	±, —
Brunner's glands	—	±	±	+
Colon	—	+, ±	±	±, —
Larynx	-	±	±	±, —
Bronchioles	—	+	±	—
Bile ducts	—			—
Gallbladder	±, —	+	+, ±	—

Table 19 completes the miscellaneous analyses which we did. Lipase is present in surviving acinar cells in patients with fibrocystic disease in apparently normal amounts. The sulfhydral and disulfide contents shows no differences, which may indicate the presence of normal quantities of chymotrypsinogen. Acetylation is a procedure that will block the oxidizing reactions of the mucuses The acetylating ability of both groups is about the same

Table 18

Digestibility of mucins in vitro

	Pepsin 3 hrs.	Pepsin 20 hrs.	Trypsin 3 hrs	Trypsin 20 hrs
Controls				
Pancreatic ducts	±	± to —	D*	D
Stomach	++	+	++	+
Small intestine	+	±	D	±
Brunner's glands	+	±	D	—
Colon	±	±	—	±
Larynx	±	—	D	D
Bile ducts	±	—	D	D
Pancreatic fibrosis				
Pancreatic ducts	+ to ±	±	± to —	± to —
Stomach	++	±	±	±
Small intestine	++ to +	± to —	+	+
Brunner's glands	+	+ to ±	± to —	+
Colon	±	—	—	±
Larynx	±	± to —	—	—
Bronchioles	±	—	D	D
Bile ducts		—	D	D
Gallbladder	± to —	—	—	D

	Hyal 4 hrs	SKD 4 hrs	SKD 22 hrs.
Controls			
Pancreatic ducts	±		±
Stomach	++	++	++
Small intestine	++	+	++
Brunner's glands	++	++	++
Colon	±	±	±
Larynx		++	++
Bile ducts	±	±	±
Pancreatic fibrosis			
Pancreatic ducts	+++ to +	+++ to +	++ to -
Stomach	++	++	++
Small intestine	+	++ to +	+
Brunner's glands	++	++	++
Colon	±	+ to ±	+
Larynx	++	++	++
Bronchioles	-	—	—
Bile ducts	+	+	+
Gallbladder	±	±	±

* D means sections totally dissolved

Role of Staphylococcus

DR. VERNON KNIGHT: There has been a considerable lack of appreciation of what is meant by the term "a pathogenic staphylococcus." Because there is no good test of pathogenicity in animals, such as one finds available with the pneumococcus, pathogenicity of the staphylococcus is defined bacteriologically. The test that will distinguish most consistently the disease producers from the nondisease producers is the coagulase test, indicating pathogenicity in the sense that coagulase-positive strains most commonly are isolated from cases of staphylococcus infection.

At least four hemolysins have been separated from the staphylococcus, but the one that exhibits the most complete hemolysis on the blood agar plate is alpha hemolysin. It correlates very well with production of coagulase, and with virulence, as estimated by isolation from cases of human infection.

Another characteristic of importance is the fermentation of mannitol, also fairly reliably associated with the pathogenicity of staphylococci. Still another test which indicates potential pathogenicity is the suspectibility of staphylococci to bacteriophage. Staphylococci that produce coagulase can be phage-typed, those that do not produce coagulase cannot.

Staphylocci can be divided into three groups by phage type, which correspond extremely closely with the three serologic types discovered by Cowan.

The staphylococcus isolated from outbreaks of food poisoning and from pastries and contaminated materials generally exhibits, in addition to these characteristics, a capacity to liquefy gelatin. It has not been observed that the specific toxins elaborated by staphylococci in artificial media play an important role in the symptomatology of the infection in man.

Role in cystic fibrosis

The relationships between pathogenic staphylococci and cystic fibrosis comprise a field where speculation must hold because there are few facts available which provide information on the problem. Though often present in the throat we would not expect chronic infection with the pneumococcus or streptococcus, because these organisms stimulate an immune response sufficient to terminate infection. The gram-negative bacilli are in a minority in cultures of the nose and throat and therefore apparently not usually available to cause respiratory infection

Summary and conclusions

From the 1600 slides studied to date, we have a few hints that the mucins of patients with cystic fibrosis may contain more numerous weakly acidic groups; they may be slightly more resistant to tryptic digestion; they may have some kind of a bond which is more easily broken by sulfuric acid-permanganate.

We can do very little in histochemistry to characterize the protein moieties of these mucoproteins, and we therefore cannot say that they are not abnormal. At any rate, studies on these protein moieties should be pursued Our largely negative results may have some value in indicating aspects of mucoprotein chemistry where any differences, if they exist, do not seem to lie

There are many enzymes which might possibly be involved in abnormal mucus formation, if such, in fact, exists. Carbonic anhydrase is one that might be involved in the sweat gland dysfunction. Preliminary studies have shown that the carbonic anhydrase inhibitor, Diamox®, does not alter the electrolyte concentration in sweat toward the pattern seen in fibrocystic disease.

Discussion

DR DI SANT'AGNESE: We have made one biopsy of the pancreas at a time when the patient had apparently normal pancreatic function. There was no evidence of obstruction of any kind, but there were inflammatory changes.

I should like to raise the question of the frequency of involvement of the bile duct, it has been said that some involvement is present in all patients.

DR. LANDING: The lesion in the pancreas is not advanced to the same degree in all areas at any one time. Furthermore, in any given area there is a slowly progressing lesion, as shown by the number of patients who have been studied clinically, who show progressive loss of tryptic activity in the duodenal fluid A normal area of pancreas cannot be taken as representative of the whole

I do not know whether the basic trouble lies in the ducts or acini, which is really the root of the problem They both seem to be affected in established lesions In newborns with meconium ileus, the acini seem to be affected first, because plugs can be seen in acini, without obvious plugs in the ducts

While the biliary tree is much more commonly affected than has been generally thought, a significant degree of pathology is quite rare

studies at Nashville and New York City. There is a similar degree of resistance to several of our commonly used drugs These resistant strains were predominately group III phage reactors.

Although staphylococci were apparently not natively highly susceptible to chloramphenicol, they seem to have less capacity to become resistant to it *in vivo. In vitro* we can unquestionably demonstrate emerging resistance to chloramphenicol after prolonged exposure This is one of the drugs we believe to have a definite place in the treatment of staphylococcal infection

Table 20

Antimicrobial susceptibility of staphylococci

New York, 1953-54—516 strains
Nashville, 1955 —647 strains

	Penicillin percent		Tetracyclines percent		Streptomycin percent	
	N. Y.	Nash	N Y.	Nash	Nash.	N Y
Resistant	72	58	66	51	66	52
Intermediate	13	21	1	2	33	45
Susceptible	15	20	33	48	1	3

	Chloramphenicol percent		Erythromycin percent	
	N Y	Nash.	N Y	Nash.
Resistant	9	1	1	2
Intermediate	86	91	2	6
Susceptible	5	8	97	92

Erythromycin is not used widely, therefore, a high proportion of strains of staphylococci are still highly susceptible to this drug

We found that patients who received no antibiotics during hospitalization had less tendency to acquire the drug-resistant strains in contrast to patients treated with tetracyclines and penicillin This represents an astounding influence of medication in an epidemiologic situation. I do not know of any natural situation where differences in the rate of exchange of bacterial flora are so remarkably distinct

In early studies there was no predominance of group III phage types in the drug-resistant strains, and the organisms were mostly

The staphylococcus will grow almost anywhere, grow well through a wide range of pH and in the presence of many substances that interfere with the growth of other organisms. A medium containing 7.5 percent sodium chloride is effective in isolating staphylococci from mixed cultures. This suggests that something in the salt concentration of the pulmonary secretions might be favorable to the growth of staphylococcus, such as has been described in the sweat and saliva in patients with cystic fibrosis of the pancreas.

This parasite produces a different type of inflammatory reaction in tissues than does any other organism. Although staphylococci produce agglutinating antibodies when they infect humans, there is no durable immunity. With a staphylococcic infection, there is little or no impetus on the part of the body to cure itself, as far as can be determined with present methods.

In the symbiotic relationship which develops in patients with chronic pulmonary disease and staphylococcal infection, the flare-ups come along, but the staphylococcal infection tends to remain localized in contrast to infection with highly virulent group A streptococci. Thus, this organism fits well the situation of cystic fibrosis.

Our evidence suggests that, although we are now able to distinguish among pathogenic staphylococci with our phage-typing efforts, any one which produces coagulase and has these other characteristics has the capacity to produce infection in man. There does not seem to be a specific sub-group of staphylococci which can be implicated in this disease.

It is likely, if there is some factor at work such as heavy exposure to one kind of staphylococci or treatment with a particular drug, that these patients will respond with changes in staphylococcal flora in much the same way as do normal people who are carriers of staphylococci.

Epidemiology

In a recent study of adult patients on a hospital ward, cultures of the nose and throat were obtained every day for five months. The results show that there is a considerable uniformity in the carrier rate of pathogenic staphylococci in a hospital, which corresponds with scanty reports from other centers. The mean rate of positive cultures in this five and one-half-month period was 19.5 percent. The throat proved to be less often positive for pathogenic staphylococci than the nose.

Antibiotic treatment of these patients did not alter the staphylococcal carrier rate, possibly because of the high resistance to drugs of the staphylococci present in the hospital. Table 20 show the sensitivity studies of the strains of staphyloccoccus which we cultured in

Metabolic Studies of Fibrocystic Disease of the Pancreas

DR CHARLES U. LOWE: We have correlated and compared the data from 44 published metabolic study periods from patients with fibrocystic disease, with those of about 70 periods from normal patients in various age groups. Statistical treatment provided several conclusions.

We could find no evidence that a high intake of fat had any effect on absorption of nitrogen (figure 12). The attitude that it is

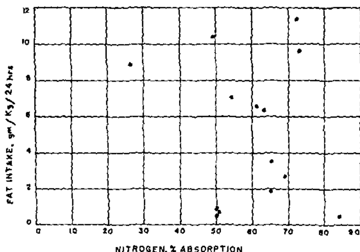


Figure 12. Relation of nitrogen absorption to fat intake in patients with fibrocystic disease on normal diets

harmful to feed a child with cystic fibrosis of the pancreas a high fat diet appears to be unsupported by experimental evidence. There was an excellent correlation between fat intake and fat absorption.

When the effects of specialized diets that included protein hydrolysates or pancreatin were compared with the effects of normal diets, in children with fibrocystic disease, there was a statistically significant difference in the nitrogen retention between the two

drug-susceptible; these observations were made before antimicrobial drugs were available.

Summary

In hospital populations the use of antibiotics can change the rate of acquisition of drug-resistant strains of staphylococci. There *is sound epidemiologic evidence that this is truly acquisition and* not mutation within the patient. Acquisition may be modified materially by choice of antibiotic drugs. Prolonged use of a drug such as tetracycline will certainly guarantee the development of drug-resistant strains, at the same time probably gradually interfering with the therapeutic result. This poses enormous problems in therapy of staphylococcal infections because of their chronic nature. We must learn to alternate our drugs, and hope for new antibiotics to replace those to which the organism has become resistant. I prefer to give patients no more antimicrobial treatment than necessary

Discussion

Dr. Paul R. Patterson: Does Knight think it would be worthwhile to isolate patients who have a diagnosis of cystic fibrosis and have been on antibiotics for a period of time, or any patients who have been on antibiotics, for the protection of surgical patients and children in the hospital?

Dr. Knight: That question is coming up more and more, but it is not so much a matter of isolation as it is of drug treatment Obviously, a patient under treatment with one of the major drugs presents a difficult situation

I do not know of any very satisfactory isolation procedure. someone has to handle the patient Even in taking throat cultures strains may be transferred from person to person

Dr. di Sant'Agnese Some patients who are said to have resistant organisms on the basis of tests of penicillin or streptomycin sensitivity actually improve clinically when large doses of the antibiotic are given

Dr. Knight: We must realize that tests *in vitro* demonstrate only a limited facet of the characteristics of drug resistance, and there is no doubt that benefit can be gained by increasing dosages in special situations seen, in face of resistance *in vitro.*

Respiratory Acidosis — Basic Physiology

DR. ERIKA BRUCK: Respiratory acidosis has been seen in some patients with cystic fibrosis of the pancreas. It is due to the retention of metabolically evolved carbon dioxide, which disturbs the ratio between bicarbonate and carbonic acid in the blood and alters pH when the retention becomes acute. In chronic cases, where the arterial retention of CO_2 is high, retention of bicarbonate also occurs, and the pH may be close to normal.

Figure 13 is a diagram arranged so that on the horizontal axis the arterial CO_2 tension can be read in millimeters of mercury. In

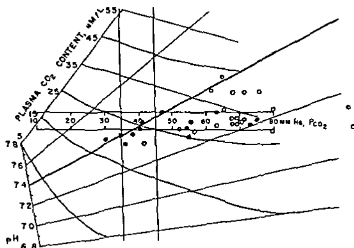


Figure 13. Data obtained from patients with cystic fibrosis showing the prevalence of some degree of respiratory acidosis.

this diagram the points represent all the data obtained in our patients with cystic fibrosis of the pancreas, and indicate that most have some degree of respiratory acidosis.

Mechanisms

Table 21 lists the basic causes of respiratory acidosis. An increase or decrease in alveolar ventilation determines the oxygen

groups of patients. The predictability of nitrogen retention is the same, whether these children are given normal diets or specialized diets, with or without pancreatin. Caloric intake and nitrogen retention show a high degree of correlation in these patients. Nitrogen intake and nitrogen retention show a poor correlation.

At constant caloric intake, there is no predictability of the relation between nitrogen intake and nitrogen retention. If the nitrogen intake is kept constant, there is a high and significant degree of correlation between caloric intake and nitrogen retention.

For comparison with normal patients, we lumped together all data from children with fibrocystic disease regardless of type of diet, but separated them according to age, using two years as the dividing point. Most of the balance material available on infants was for under one year of age, but there were two published studies in children at 19 and 22 months of age.

We found that children with fibrocystic disease of the pancreas have the same degree of predictability of nitrogen retention with a given intake of calories or nitrogen as do normal children, with one important exception. Plotting the predictability shows that as either caloric intake or nitrogen intake drops in these patients, they go into negative nitrogen balance at a higher intake than do normal children. Comparison with studies on undernourished children showed that they behave much like children with fibrocystic disease of the pancreas, in that they have an avidity for nitrogen.

Discussion

DR. ZUELZER: Is there animal experimental data that would show a similar nutritional relationship between animals with ligation of the pancreatic ducts or other forms of pancreatic insufficiency, and normal animals?

DR. LOWE: Papers by Handelsman, Greenberg, Bowman and Mann have shown a similar correlation between caloric intake and nitrogen retention.

fusion The alveolar CO_2 and arterial CO_2 tension are usually not changed.

In some cases there may be decreased arterial CO_2 tension and respiratory alkalosis because, if there is sufficient anoxia to stimulate

whether the shunt occurs in the lungs or in the heart.

When the distribution of gas is uneven and when diffusion is impaired, alveolar oxygen may be high, especially if there is hyperventilation. There is considerable difference between the alveolar oxygen tension and the arterial oxygen tension, i e., the alveolar-arterial oxygen gradient is increased.

With hypoventilation, i e, decreased alveolar ventilation, the alveolar oxygen is low, and the gradient between alveolar and arterial oxygen should be normal. Hypoventilation is the outstanding mechanism in the production of respiratory acidosis.

Causes of hypoventilation

I have listed in table 22 the causes of decreased alveolar ventilation. Chief among these is depression of the respiratory center.

Table 22

Causes of decreased alveolar ventilation

1	Depressed respiratory center
2	Paralysis of respiratory muscles
3	Decreased thoracic space or mobility
4	Increased intrathoracic pressure
5	Decreased distensibility of lung
6	Increased respiratory dead space
7	Obstruction

Some respiratory physiologists believe that there is no respiratory acidosis without depression of the respiratory center

Increased intrathoracic pressure is also an important cause of hypoventilation, because air can not move against a pressure gradient The outstanding example of this is found in tension pneumothorax

and CO_2 exchange. Minute ventilation or tidal volume does not give a good indication of whether ventilation is adequate. The effect of hypoventilation or decreased alveolar ventilation is more marked on CO_2 tension than on that of oxygen. This has to do with the fact that oxygen diffuses in the gaseous medium of the airways more rapidly *than CO_2. When diffusion through the alveolar* membrane is impaired, as in pneumonia or pulmonary emphysema, there is little effect on the arterial CO_2 tension, because the diffusion *coefficient*

Table 21

Effect of functional derangement on blood gas tension

Abnormality of Ventilation	Pa_{CO_2}	Pa_{O_2}
Alveolar ventilation increased	↓	↑
Alveolar ventilation decreased	↑	↓
Diffusion impaired	—	↓
Distribution of gas uneven	— or ↑	↓

of CO_2 is about 20 to 25 times as high as that for oxygen, and the patients develop marked decrease of the arterial oxygen tension long before there is any effect on CO_2

Distribution of gas in the lungs is an important factor If there is only one lung with normal ventilation, and it is well perfused with blood, the blood oxygen and CO_2 will be normal However, if there are two lungs, only one of which is ventilated as before, but both lungs are perfused with blood, blood will return from the nonventilated lung and mix in the pulmonary veins with the blood from the normal part Theoretically the oxygen saturation in the mixed blood will be low and the CO_2 tension high

The CO_2 in these cases, however, is usually not affected because blood from the pulmonary vein stimulates the respiratory center causing hyperventilation of the well-ventilated lung The CO_2 tension in the blood coming from the good lung can thereby be diminished to levels of 30 or 15 mm of mercury When mixed with blood from the nonventilated part of the lung, the CO_2 tension in the mixture may be normal This cannot happen with oxygen because the hemoglobin in the blood perfusing the normal lung is saturated to almost 100 percent and cannot take up any more oxygen

Cardiac and Pulmonary Complications in Fibrocystic Disease of the Pancreas

Cardiac complications

DR. STEPHEN W. ROYCE: Our experience with cardiac complications in fibrocystic disease is limited to the rather poorly understood phenomenon of cor pulmonale. Approximately one-third of the children who die with fibrocystic disease show, at some time, premortem evidence of clinical heart failure The lives of these children are usually terminated in an acute episode of irreversible heart failure

The chest findings at postmortem are such that evidence of pulmonary heart disease might also be expected. Characteristically, there are an emphysematous chest and hyperexpanded lungs that do not collapse when they are cut The diaphragm is low and flat. All these factors predispose to right heart strain The heart is usually small in bulk, but above normal in weight The anterior wall of the right ventricle is usually thicker than normal, a sign no longer considered a reliable index of right ventricular hypertrophy.

Cardiac catheterization studies

Since the physiologist regards a mean pulmonary artery pressure of 25 mm or more of mercury as the essential criterion for right heart strain, it was arranged to subject 11 of our patients to cardiac catheterization studies

The patients ranged in age from 4 to 10 years All had varying degrees of pulmonary insufficiency as determined by auscultation, roentgenographic study and exercise tolerance, and all had a chronic cough. The studies we performed were standard for cardiac patients, with the catheter introduced into the left arm, thence through the right heart and into the pulmonary artery.

The correlation between high mean pulmonary artery pressure and low arterial oxygen saturation is shown in table 23.

Pulmonary heart disease occurs in some children with fibrocystic disease and, in general, the degree of right heart strain is in direct proportion to the degree of lung involvement. Whether right heart strain is a dominating factor in determining the prognosis in fibrocystic disease is still unknown.

Obstruction is a possible cause of hypoventilation. However, there are practically no reports of respiratory acidosis described as caused by hypoventilation due to obstruction. Respiratory acidosis is characteristically absent in asthma.

Respiratory acidosis in cystic fibrosis

Since obstruction is described as the primary and most characteristic anatomic lesion in the lungs of patients with cystic fibrosis of the pancreas, it seemed worthwhile to compare the evidence for obstruction and hypoventilation in these patients, as measured by the pneumotachograph, with results of determination of gases in arterial blood.

Six patients were studied and all had evidence of obstruction, by pneumotachographic analysis. Determinations of gases in the blood show that slight or moderate obstruction does not produce sufficient hypoventilation to cause CO_2 retention, only three of the patients had respiratory acidosis, but all had reduced blood oxygen tension. The lowering of oxygen tension in arterial blood is apparently not caused by hypoventilation. In fact, the alveolar-arterial oxygen gradient was increased in all these patients, probably because of diffusion difficulty or uneven distribution of gases.

Since two patients had no pneumonia, exudate or bronchitis at the time they were studied, there was no reason to assume an anatomic barrier to diffusion in these cases. We can postulate, therefore, that most of the patients had uneven distribution of gases, probably due to atelectatic areas, or possibly due to collateral circulation in the lungs, shunting blood away from the right to the left side of the heart.

It has been noted that adults with respiratory acidosis show depression of the respiratory center and, therefore, breathe mainly under the stimulus of the reflex centers which are stimulated by anoxia. If you remove the anoxic stimulus by giving these patients oxygen the results may be disastrous.

We have done only two preliminary studies on the effect of oxygen therapy in patients with fibrocystic disease. Our findings corroborate those from studies on adults that respiratory acidosis can be aggravated by oxygen therapy because ventilation is decreased and CO_2 tension increased. Clinically, we have not seen adverse effects of administration of oxygen and I would not advise withholding oxygen from these patients although we must be aware of its potential danger. Adults with emphysema have been treated with combined artificial respiration and oxygen.

found evidence that exercise tolerance is impaired, and some have clubbing of the fingers, even in the absence of any other signs of lung disease Our experience suggests that eventually they will find their lives dominated by the state of disease in their lungs.

McIntosh, in studying a group of children over 10 years of age, found that all reported some coughing, even though their early childhood had been characterized primarily by gastrointestinal disturbance, with little coughing during the first year of life.

Correlation of age with the onset of cough in some 300 cases is shown in figure 15. Most patients begin to cough in the period before six months of age

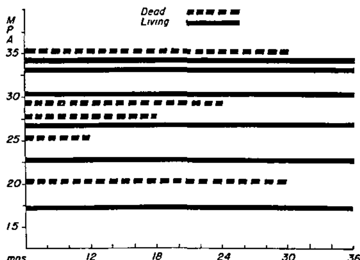


Figure 14 Length of life compared with mean pulmonary artery pressure No correlation is seen

Before antibiotics were available, the survival of a child who coughed during the first weeks of life was generally measured in terms of months Of 38 infants seen with persistent cough before the age of two months who were treated with antibiotics, one-third were dead within six months. Despite antibiotics the early onset of cough continues to carry a poor prognosis, although survival time has been lengthened.

Coughing suggests that the earliest problem is one of intra luminal obstruction Relief of obstruction is the objective of coughing as well as of the therapeutic regimen The effectiveness of therapy is often assessed by the degree that coughing is relieved

Figure 14 shows a three-year followup of the original studies. The lack of correlation is apparent. The usefulness of cardiac catheterization as prognostic tool would appear to be limited.

The studies showed, however, that there is laboratory evidence of right heart strain, which does correspond, roughly, to degree of pulmonary insufficiency. It does not have a consistent relationship with the shortened length of life.

The pathogenesis of cardiac failure in cystic fibrosis disease makes it unlikely that any therapy would have more than limited effectiveness Digitalis and mercurials provide only temporary relief. Cardiac failure may be reversible, but only for a matter of weeks It is a sign that the lung field has lost its elasticity. To the handicap of pulmonary hypertension is added the handicap of alveolar congestion with increased pulmonary rigidity. In our experience there is no sign that has worse prognostic import than the onset of congestive failure

Table 23

Relation of pulmonary artery pressure to systemic oxygen tension

Pulmonary artery pressure		Arterial oxygen saturation
systolic	mean	
24	18	84
30	20	84
40	25	84
33	25	86
41	26	85
43	27	86
34	28	82
40	31	89
45	35	77
42	35	74
44	35	71

Effect of pulmonary disease

The general health of children with fibrocystic disease, their appetites, activities, and longevity are closely correlated with their degree of lung involvement

Approximately five percent of these children do not cough and do not have chronic respiratory disease. Even among these we have

The "ventilation factor" is an average of the timed vital capacity, the maximum breathing capacity and the residual capacity expressed as percentages This roughly approximates the effectiveness of the lungs as a bellows mechanism and gives some measure of pulmonary dysfunction. The ventilation factor in this patient was 38 percent of normal, which means there is emphysema, with a tremendous amount of air trapping

Table 24

Pulmonary function studies

	Predicted	Observed	% Normal
Lung volume measurements			
Vital capacity, ml	3136	1664	53
Timed vital capacity, 3 sec ml	3136	1292	41
Max. breathing capacity l/min	99	29 8	30
Max.—after bronchodilator l/min	99	51	52
Residual capacity			
Residual air capacity, ml	1 5	2.3	
Total lung capacity, ml	784	1482	190
Residual capacity of total lung	3920	3146	80
capacity, ml	20	47	
Ventilation factor, percentage	100	38	38

Evaluation of pulmonary disease

The extent and severity of the pulmonary disease can be approximated using the ratings shown in table 25 This technique affords means of charting changes and making comparison of the patient with other children

Principles of therapy

Unless started before coughing begins, we should regard therapy in fibrocystic disease as active, not prophylactic treatment There are two prophylactic measures, both surgical, that may be indicated after onset of pulmonary symptoms.

It has been shown that in heart failure, rales are often more pronounced on the right side of the chest than the left. Long before heart failure occurs, however, the child with fibrocystic disease is likely to show auscultatory evidence that the right side of his chest is more severely involved than the left. The syndrome of lobar atelectasis involves most frequently the right upper lobe, then the

Once the cough becomes persistent, progressive involvement is the rule. Evidence that the child is losing the ability to ventilate his chest increases. Coughing spells are attended by flushing, then cyanosis, and, finally, by complete exhaustion. The chest movements become more rapid but also less efficient. Then the chest becomes fixed in a position of full inspiration, and respiratory activity is limited. The terminal phase is that of severe emphysema, accompanied by a deep, wet, ineffective cough that exhausts the patient but does not produce sputum. The data in table 24, taken from observations on one child, are representative. The administration of a bronchodilating agent increased her ability to ventilate the chest to a significant degree.

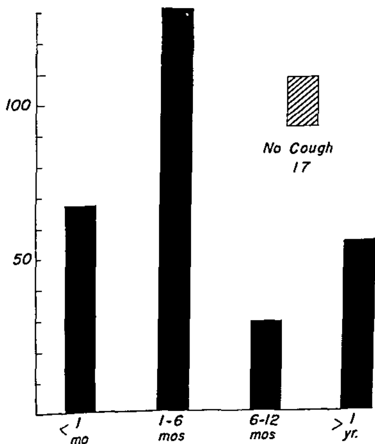


Figure 15. Age of onset of cough in 300 patients with cystic fibrosis of the pancreas

In advanced lung disease there is evidence that a barrier develops between the alveolar wall and the blood stream, and that antibiotics given by mouth or injection do not reach the site of infection. Here the use of aerosol therapy may be effective.

Table 26 lists several aerosol mixtures. Zephiran®, in combination with streptomycin and penicillin, is particularly effective. We have limited experience with Tryptar®. Our impression is that it is an extremely irritating material when given at the recommended rate. Of all the combinations used, that of the bronchodilator, Isuprel®, and Terramycin® is, perhaps, the most effective.

Aerosols are most effective when the particle size is within a critical range of .05 to 3 millimicrons in diameter. The Bennet mask, the DeVilbiss No. 40 nebulizer and the Vaponefrin® nebulizer have been used satisfactorily. The DeVilbiss 501 is a satisfactory compressor.

Table 26

Aerosol agents used as mixtures

Zephiran®	"Mistogen Solution"	
Tryptar®	Ephedrine Alkaloid	0 8 Gm
Dornase®	Propylene Glycol	25 cc
Allevaire® (Triton A-20)	Glycerine	25 cc
Isuprel®	Distilled water ad	1000 cc
< Antibiotic Combinations >		

We use aerosols, 1 5 ml of solution, for 20 minutes three times a day, and usually try to follow with a period of postural drainage This technique is practical for use with young children.

As respiratory involvement increases, the effectiveness of aerosol therapy decreases rapidly. In the fixed emphysematous state, aerosols probably penetrate to, but not beyond, the site of obstruction Poorly ventilated or nonventilated areas of the lung receive no therapeutic benefit To reach these areas, more complex equipment, such as the intermittent positive pressure breathing machine, must be used Physiologic evidence suggests that this type of therapy may possibly arrest the progression of emphysema in children

Lacking knowledge of the specific defect in the lungs, we are faced with the necessity of treating signs and symptoms as they arise, rarely being able to anticipate or judge the effectiveness of our

right middle lobe, and rarely if ever involves the left side of the chest. Since prognosis in this syndrome is poor, we might expect that resection of the lobe or part of the lobe would be followed by improvement.

Eleven of 23 patients examined at more than 10 years of age showed an involvement of the paranasal sinuses. Polypectomy

Table 25

Clinical rating

	5	4	3	2	1
Exercise tolerance	normal	sl. limited	sev. limited	home	zero
Cough	absent	rare	1 session daily	constant	constant & ineffective
Breathing	eupnea	hyperpnea	dyspnea $\bar{c}$ exertion	dyspnea at rest	orthopnea
Clubbing	absent	angle reversed	moderate	severe	severe with cyanosis
Chest Auscultation	clear	coarse breath sounds	scattered rales	generalized rales	sticky

or other treatment of the nasal sinuses might also be considered prophylactic therapy.

Other therapeutic measures seem entirely active and should be planned for home care rather than hospital care

Antibiotic therapy

A difference in survival time following the use of penicillin in fibrocystic disease has been demonstrated. An even greater increase in survival time will follow the use of the newer antibiotics, preferably used in combination and alternation Dosage should be large 30 to 50 mg/kg per day

Postural drainage

With an efficient cough the patient removes debris consisting of bacteria, mucus and casts of the bronchioles Therapy must increase the productiveness of the cough. Placing the patient in a jackknife position, with pillows under his abdomen, and leaving him for twenty or thirty minutes, and placing blocks under the foot of the child's bed to maintain bronchial drainage during naps and during sleep are effective measures for bringing up sputum

Genetics

DR. BARTON CHILDS: Considerable evidence has accumulated suggesting that cystic fibrosis of the pancreas is a genetically determined condition. Affirmation of a single gene hereditary characteristic in humans depends, in part, on agreement of observed distributions of affected and unaffected individuals within family groups of various sizes, with distributions expected according to specific hypotheses of modes of inheritance of genes.

In most instances, the particular mode of inheritance will depend on the choice of criteria by which it is determined whether or not an individual is affected. Thus, whether we consider a gene as dominant or recessive depends on whether we can detect both heterozygote and homozygote. A dominant gene is one that will produce equal effects in both heterozygote and homozygote. It seems that many so-called dominant genes do not fulfill the definition of this term, because the manifestation in homozygotes is unknown. It is probable that every gene has a function that can be measured if detected, and we may escape the rigidity inherent in the use of the terms dominant and recessive if we consider what genes do in the homozygous and heterozygous states.

When the genetics of cystic fibrosis of the pancreas was first studied, it seemed that the disease was due to a gene which expressed itself only in the homozygote, a perfect recessive. This assumed rather rigid criteria for diagnosis of the disease, including in most instances marked reduction or absence of pancreatic enzymes as well as pulmonary disease, or diagnosis at autopsy. Assuming the proposed gene to behave as an autosomal recessive, statistical agreement has been demonstrated in several studies between the number of patients expected in families and the number observed.

Table 27 shows the results of an analysis by the method of Hogben of data pooled from various investigations. There is no significant departure from expectation in the total values, nor is there a difference [illegible]

the distribution within families that we see in these data.

therapy. It seems clear that therapy aimed at the relief of intraluminal obstruction must be given continually and for long periods of time, and must be planned as home therapy.

Discussion

DR. DI SANT'AGNESE: The cardinal and primary occurrence in the lung in fibrocystic disease is bronchial obstruction leading to generalized obstructive emphysema. With obstruction, secondary bronchopneumonia occurs. If the infection continues for months or years there may be permanent damage to the bronchi and progressive bronchopneumonia which is not checked by antibiotics. This leads to pulmonary insufficiency and death.

As long as there is no irreversible damage to the bronchi, antibiotic therapy is often successful in checking the process.

DR ANDERSEN: We have been discouraged with the results of postural drainage. In contrast, we find there is some benefit in allowing the children to be up and around, at least for intervals during the day. It seems that in the latent stage of this disease, activity is more effective than postural drainage.

Our experience with lobectomy includes seven children, two of whom were operated on at other hospitals. We believe that there is a limited number of patients for whom this operation is appropriate. Our criteria for lobectomy are as follows:

1) that the segmental atelectasis has been localized for at least six months;

2) it involves not more than two lobes, usually the right upper and middle,

3) the other lung must be free or nearly free of active infection following antibiotic therapy. We have observed spontaneous re-expansion of the atelectatic lobe following antibiotic therapy, sometimes several months after its discovery. The patient should therefore be given a trial of this therapy before surgery is seriously considered.

among individuals who could be heterozygotes. Since this time, parents and sibs have been found showing either high sweat electrolyte values alone, or in combination with obstructive emphysema and chronic bronchopneumonia. It appears that not all individuals [illegible]

that many of these show values of sweat electrolytes which are somewhat lower than those found in fully affected patients.

Hypothetical mechanisms

Though we assume the disease to be caused by a mutant gene or genes, which affect a wide variety of organs, we do not know the precise mechanism through which such a gene or genes act. Nor do we know the causes of variation in the expression of such genes in patients and their relatives. The question arises whether there might be more than one genetic entity, that is, several genes operating in different ways For instance, there might be two or more genes at different loci, not interacting, and whose modes of action are quite distinct, although producing certain phenotypic similarities. This might account for differences between families in sweat electrolytes and pulmonary disease among possible heterozygotes

Alternatively there might be a system of multiple alleles, genes occupying the same locus, but whose actions are quantitatively different Finally we must consider the effects of genotypic or environmental modification of the expression of such genes. It is well known that the phenotypic expression of single genes is subject to modification by other genes in the genotype, and that such expression depends upon the environments in which the gene acts These hypotheses might account for some of the variability in the clinical manifestations among homozygotes, in which all the exocrine glands may be involved, or different ones in various degrees They might also account for the fact that some probable heterozygotes may show not only sweat electrolyte abnormalities, but also chronic pulmonary changes, while others exhibit only some alteration in the sweat Thus parent-child combinations showing partial forms of the disease in probable heterozygotes may be due to a gene which in such families could be called incompletely dominant This phenomenon is seen in patients with sickle cell disease who have thrombotic phenomena in the presence of electrophoretic patterns showing both normal and sickle cell hemoglobins. It is compatible with what is known of the action of natural selection, which works toward suppression of the effects of deleterious genes, causing them to evolve in the direction of recessiveness

Other criteria must be fulfilled to provide support for this hypothesis. Males and females should be equally affected, and this was found to be so. No affected parents were noted. Of 38 step-children studied none were affected. If we assume the disease to be found among heterozygotes, approximately one-quarter of the step-children should be affected. If, on the other hand, the disease is found only among homozygotes, the probability of finding affected individuals among step-children would be one-quarter the probability of the common parent marrying another heterozygote

Table 27

Siblings in family	No. of families	Total siblings	Correspondence of expected and observed distribution of affected individuals within families		
			Observed	Expected	Variance
1	67	67	67	67	0 000
2	64	128	70	73 14	7 700
3	65	195	90	84 31	17.095
4	22	88	36	32.18	9 240
5	13	65	25	21.31	7.700
6	3	18	6	5.48	2.328
7	5	35	12	10.10	4 850
8	1	8	1	2.22	1 172
9	1	9	1	2 43	1 380
10	2	20	7	5 30	3.184
	243	663	317	303 50	54 739

$S E = \sqrt{54.739} = 7.40$
$D = (317 - 303.50) = 13.50$

$$\frac{D}{\sqrt{S E}} = \frac{13.30}{7.40} = 1.82$$

There were also a few affected collateral relatives, a phenomenon we would expect, since, if the disease appears only in homozygotes, the incidence among collateral relatives should be several times that among all the population, though still manyfold less than within immediate families.

Statistical evaluation alone does not prove heredity, and the hypothesis would be founded upon firmer ground if we could find a characteristic among parents and other relatives which would satisfy mendelian requirements. Until the discovery of the elevation in sweat electrolytes among some of the relatives of affected patients, there was no published evidence of any effect of the presumed gene

among individuals who could be heterozygotes. Since this time, parents and sibs have been found showing either high sweat electrolyte values alone, or in combination with obstructive emphysema and chronic bronchopneumonia. It appears that not all individuals who may be presumed to be heterozygotes show abnormality. In fact, the data of di Sant'Agnese indicate that the majority of such individuals are clustered within a few families. It may be significant that many of these show values of sweat electrolytes which are somewhat lower than those found in fully affected patients

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6	3	18	6	5 48	2 328
7	5	35	12	10 10	4 850
8	1	8	1	2 22	1.172
9	1	9	1	2 43	1.380
10	2	20	7	5.30	3.184
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sweat electrolytes among some of the relatives of affected [illegible] there was no published evidence of any effect of the presumed gene

expression of the disease in homozygotes, but in only partial expression in some heterozygotes. Thus we may say that in most families the recessive hypothesis is fulfilled, while in some the gene may be called partially recessive or partially dominant.

Discussion

DR. ROYCE: The statement has been made that this is the most common lethal gene in the population. Is that true?

DR. CHILDS: It may be so. The incidence of the disease has been estimated to be between one per 1,000 and one per 10,000. Assuming no selective advantage for the heterozygote, an assumption which may not be justified, the calculated mutation rate would be one per 1,000 to one per 10,000 mutations per locus per generation. This is a situation quite outside our experience with any other human disease, and for this and other reasons this calculation must be considered with much reservation. Most mutation rates have frequencies less than one per 25,000.

DR. DI SANT'AGNESE: Childs has appropriately emphasized the variability of expression of genes. With regard to the sodium and chloride concentration in sweat we have taken 60 mEq/l of sodium or chloride as the upper limit of normal, because the concentrations in all our patients with known cystic fibrosis were above that. There is no reason why heterozygotes should not have slightly lower concentrations. However, electrolyte concentrations vary so much in apparently normal individuals that it would be hard to recognize the abnormal.

DR. CHILDS: There is no reason at all why the sweat abnormality cannot be as variable as anything else. However, the closer we get to measuring the fundamental gene action, the less modification or variability we find in the expression of that characteristic in those individuals who hold it.

DR. SHWACHMAN: I should like to bring up a point that has been raised in early investigations, that perhaps this disease is in some way related to one of the blood groups. This is not the case. We thought with Steinberg it would be worthwhile to investigate the possibility of a genetic linkage between the blood groups and this disease, because the hereditary pattern of cystic fibrosis is simple and known. Analysis of data from 70 families uncovered no relationship between cystic fibrosis and any of the following blood groups, ABO, Rh, MNS, Kell, Duffy, Lewis and P, and a few others that are not common.

The most likely hypothesis, then, is that which calls for a gene or genes which cause the disease in homozygotes and cause partial expression or no expression in heterozygotes.

Though there is insufficient published data to favor strongly either the assumption of multiple alleles, or its alternative, genes at more than one locus, it should be possible to test these hypotheses by means of measurement of sweat electrolytes in parents, sibs, and collateral relatives In general, assuming genes at different loci, both parents of a particular family and some of their relatives should show the same expression in terms of sweat electrolytes. If, on the other hand, a system of multiple alleles is responsible, we should find not two, but three types of family; those in which both parents and their relatives show the same kind of expression, as well as some families in which one parent and some of his relatives show a manifestation which the other does not

Genetic significance of abnormalities in sweat

Speculations on the genetic aspects of this disease have been based on possible distributions within families of the one common characteristic which appears in nearly every patient who has this disease, *and in some of their relatives, that is, abnormalities in sweat electrolytes Still, we find certain parents who, according to the genetic hypotheses we are considering, should be heterozygotes, but do not show the characteristics. Although the sweat electrolyte test will detect the homozygote in nearly every case, it performs this function for heterozygotes with distressing infrequency and it seems likely that this test, though the only one currently available, is not the final determinant for the presence of the gene in single dose.*

It should be possible, in theory at least, to find some quite consistent characteristic of the gene action which would expose its presence wherever it exists. This speculation is based on the assump- [illegible] measur- [illegible] asso- [illegible] n, the less modification there will be of that effect by the genotypic or external environment Such a determinant will be closely associated with whatever turns out to be responsible for alteration in function of such a wide variety of organs in the body in this disease

Summary

It seems quite likely that cystic fibrosis of the pancreas results from the effects of gene mutation There might be either a system of alleles at one locus, or genes at different loci, which result in full

DR. HERBERT J. BARTELSTONE: Aside from one short publication regarding pilocarpine and its effect of the pancreatic tissue, there has been no report of direct study of the relationship of autonomic regulation to the disease.

An avenue of research might be opened up by exploring some of the effects of electrically induced or drug-induced stimulation to some of the exocrine glands whose function is influenced by the autonomic nervous system. There might be some alteration either in the production of acetylcholine esterase or the permeability of receptor sites, which may be one of the underlying factors in the disease. Overstimulation of the parasympathetic nervous system or cholinergic fibers for a prolonged time might result in some of the findings that have been noted clinically.

Conclusion

DR. FRAZIER. From the thoughts presented here we may conclude that the genetic basis of this disease has been reaffirmed. It has not been possible to discover, by either biochemical analysis or histochemical staining techniques, a qualitative abnormality of the mucopolysaccharides in those exocrine glands which secrete mucus

A defect in production or availability of energy for elaboration of secretory products has been postulated on the basis of data obtained from study of the concentrations of electrolyte in sweat The clinical manifestations may be secondary to such a basic defect

Application of basic physiologic principles to the discussion of the deranged functions of various organs has facilitated a clear and logical exposition of sound therapeutic procedures